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*7,809 volunteer abstracts, 18 symposium and workshop abstracts.*
1988 PROGRAM COMMITTEE

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University of Iowa College of Medicine
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  E.A. Barnard | No abstract |
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**Theme G: Motor Systems and Sensorimotor Integration**
Theme H: Other Systems of the CNS

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<td>102.</td>
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<td>412.</td>
<td>Epilepsy: GABA and benzodiazepines</td>
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<td>227.</td>
<td>Epilepsy: hippocampus and neocortex I</td>
<td>Poster</td>
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<td>351.</td>
<td>Epilepsy: hippocampus and neocortex II</td>
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<td>Epilepsy: second messengers and mRNA</td>
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<td>Epilepsy: substantia nigra and amygdala</td>
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<td>New Genes from Old Diseases</td>
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No differences were observed in [\(^{125}\text{I}\)]DAGO binding in pallidum and globus pallidus. [\(^{3}\text{H}\)]QNB binding decreased processed for receptor autoradiography. Pallidum and that the mu-opioid receptors are not as projection is lesioned? Quinolinic acid or nicotinic acid pallidum respond in a similar fashion when the striatal accumbens and two weeks later, the rat brains were exhibited reduced [\(^{125}\text{I}\)-pindolol binding after IMP. Chronic imipramine treatment was also affected. Chronic imipramine (IMP) treatment was investigated with chronic IMP treatment. Subdivisions of the amygdala and hippocampus were differentially affected. Significant reductions in [\(^{3}\text{H}\)-pindolol binding in the IMP-treated rats were found in the CA-1 stratum radiatum and dentate molecular layer, but not in the CA-3 stratum radiatum of the hippocampus. In the amygdala, the basolateral nucleus exhibited reduced [\(^{3}\text{H}\)-pindolol binding after IMP treatment but the central and medial nuclei were not affected. Chronic imipramine treatment was also associated with increased [\(^{3}\text{H}\)-pindolol binding in layer 1 of the cingulate cortex and layer 3 of the piriform cortex but not in the ventrolateral thalamic nuclei, claudius-puamen, lateral hypothalamus, or layer 2 and 3 of the somatosensory cortex. The regionally selective down-regulation of beta-adrenergic receptors by chronic IMP are compared to regional distribution of binding sites for [\(^{3}\text{H}\)-DIME and [\(^{3}\text{H}\)-IMP (MESIS144 & MESIS127).
QUANTITATIVE IMMUNOCYTOCHEMISTRY USING AN IMAGE ANALYZER. II. ESTIMATION OF THE GABA-CONCENTRATION THROUGH A BIOLOGICAL STANDARD. L.B. Nabors*, E. Songu-Mize*, and R.B. Mike. Depts. of Anatomy and Neurobiology and Pharmacology, Univ. of Tennessee Health Science Center, Memphis, TN 38163

We have developed a procedure to estimate the concentration of neurotransmitters in brain based upon the optical density of immunocytochemical labeling measured with an image analyzer. A non-biological standard was used which binds conjugated neurotransmitters. Fifty micron agar sections cut from a block were used a matrix for the standard. The agar sections were activated with cyanogen bromide/acetonitrile to promote coupling to the antigen. We measured the amount of coupled antigen by counting the radioactivity conjugate. Activated agar sections were incubated in serial dilutions of the tritium labeled GABA/BSA conjugate. Some sections were then used to measure the amount of coupled antigen by counting the radioactivity with a liquid scintillation counter. The remaining sections were incubated in the GABA antibody and processed for immunocytochemistry. The optical density of these sections was measured with an image analyzer. A linear relationship was found between optical density and the log concentration of GABA over a range of at least 0.01 to 1 mmol of agar. These results suggest that the concentration of GABA within individual cells and processes can be estimated by comparing the optical density of their label with that of the non-biological standards. Supported by EY-02973 and RR-02800.


The GABA receptor complex which contains pharmacologically recognizable binding sites that interact with GABA has been the subject of considerable interest. This complex includes GABA, benzodiazepine (BZ), barbiturate, and convulsant sites that are heterogeneously distributed throughout the brain. The binding of ligands which combine with portions of this system was investigated using autoradiographic and homogenate binding techniques. Low to intermediate densities of high-affinity GABA receptors in the CNS were labeled with $[^3]H$muscimol. High densities of $[^3]H$bicuculline methiodide and $[^3]H$SR 59319 binding sites (low-affinity GABA) were found in many regions analyzed. These sites defined by $[^3]H$flunitrazepam binding were coupled to BZ receptors. BZ sites were examined using $[^3]H$-diazepam and $[^3]H$-oxazepam. Convolulant sites, which were identified with $[^3]H$TBPS or $[^3]H$BTBPS showed intermediate to high levels of binding density in those areas studied. Modulation of $[^3]H$TBPS was examined by including several BZ and barbiturate agents and varied with brain region. Our studies provide a regional comparison of the distribution and density of individual subcomponents of the GABA complex and information regarding relationships of receptor subtypes and GABAergic function.


The rat interpeduncular nucleus (IPN), a component of the midbrain limbic system, is composed of an abundant population of topographically organized GAD-positive terminals and a dense plexus of GAD-stained axons. Examination of the ultrastructural localization of GAD-staining in the IPN revealed a variety of arrangement of GAD-immunoreactive axodendritic and axosomatic synapses. The lateral, central, intermediate, and rostral subnuclei exhibited numerous GAD-positive somata and dendritic processes. Unmyelinated GAD-positive terminal-like contacts with immunoreactive dendrites and somata, while GAD-stained terminals formed symmetrical axodendritic and axosomatic contacts. GAD-positive terminal-like contacts were observed throughout the entire center forming symmetrical contacts with non-immunoreactive dendrites and somata. Immunoreactive myelinated terminal-like processes were present throughout the lateral subnucleus. This study suggests that a prominent population of GAD-positive neurons contribute to a complex intrinsic cerebellum-GABAergic circuitry, which are in receipt of numerous inputs and may give rise to a small GAD-positive projection. (Supported by the Medical Research Council of Canada).


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Calcium-binding proteins (CaBP) are intracellular proteins that translate the information carried by Ca2+ in a meaningful manner. We are interested in understanding why certain CaBP's occur only in subpopulations of neurons, and how they function as a group to deduce the function of the proteins by studying their immunohistochemical localization. This paper shows that double labeling techniques against the CaBP's parvalbumin (PV) and calbindin D-28k (28k) yield new neuroanatomical markers which not only visualize known cells and structures, but also reveal as yet undescribed nuclei and pathways. Kevelutions are highly preferentially found in a subclass of rapidly firing GABA-positive interneurons; 28k is associated with neurons susceptible to neurodegeneration. The relationship between the distribution of neurons containing these two CaBP's and that of receptors for calcium-regulating hormones and Ca2+-channels is complex. We assume that neurons containing these additional CaBP's are privileged for certain neuronal processes and suggest a role of parvalbumin in the control of neuronal excitability.


The hypothalamus is a morphologically and functionally diverse region of the brain. This laboratory utilizes dissociated cultures of embryonic rat hypothalamic tissue to investigate the expression of phenotypic properties of hypothalamic neurons in a controlled environment. In this study, we used antibodies directed against tyrosine hydroxylase (TH), GABA, and somatostatin (OMP) to investigate the time course and morphological characteristics of expression of these substances. TH neurons appear to form two populations: a large population of small 'TH' neurons which is only observed in young cultures, and a small population of large 'TH' neurons with extensive neuritic ramifications which are first observed at 8 days in vitro and can be identified in older cultures. The overwhelming majority of the neurons in these cultures appear to synthesize immunoreactive GABA; the morphology of these cells is quite heterogeneous. The fact that virtually all neurons are GABA would suggest that they contain other transmitters as well. Clusters of small neurons expressing OMP can be identified in cultures as early as 5 days in vitro. One interesting observation is that neurons which express TH and OMP are often found in groups rather than as isolated cells, which may suggest a clonal origin for these cells. Supported by NHI NS-25168 and a grant from the Foundation of UMDNJ.

315.11 NEUROPEPTIDE-Y PROJECTIONS FROM LOCUS COERULEUS TO CEREBRAL CORTEX IN THE RAT. R.J. Wilcox and J.R. Unerstall. Department of Neurology, Case Western Reserve University Sch/Med, Cleveland, OH 44106.

In a preliminary study, we have found that neuropeptide-Y (NPY) is found in cortical and hippocampal neurons and its co-localized with norepinephrine (NE) in the locus coeruleus (LC). We have mapped the rostral-caudal distribution of NPY neurons within the LC by immunofluorescence. In addition, using double-labeling techniques we have localized NE-containing LC neurons which also project to the cortex.Brains of male Sprague-Dawley rats treated first with Fluorogold, then with colchicine were fixated by perfusion. Frozen sections of LC 10 µm thick were stained for NPY-IR by indirect immunofluorescence. LC neurons labelled after cortical Fluorogold injection were found throughout the dorsal compact LC. NPY-IR neurons were distributed in the entire rostro-caudal extent of the LC including subcoeruleus (Co) and labelled neurites of these neurons containing both NPY-IR and retrograde tracer. Preliminary results show that 10% of Fluorogold labelled neurons in the rostral and caudal poles of the LC also exhibited NPY-IR. In the compact LC, a larger proportion of the Fluorogold labelled cells (up to 30%) exhibited NPY-IR. Thus, this study contributes to NPY-mediated neurotransmission in the cortex.

315.13 PMT, NPY, and SP-IMMUNOREACTIVE STRUCTURES IN THE MEDULLA OBlongata of the Tree Shrew, G. Flugi, G. Behrens, A. Hittendorf, F. Pollano* and E. Fuchs. German Primate Center, Göttingen, FRG.

Phenylethanolamine-N-methyltransferase (PMT), neuropeptide-Y (NPY) and substance P (SP) immunoreactive structures were detected in the medulla oblongata (MO) of the tree shrew, a species which provides a model of a small primate. We used double immunoperoxidase labeling against PMT immunoreactive cells (IC) corresponding to group C1 and C2 of the rat, but no group C3 were found. Most of the IC and immunoperoxidase labeling were in solitary tract nucleus (NTS) where they form a ring around the subnucleus gelatinosus (SG).

In the MO we detected very few NPY-IC but dense patterns of NPY-IR. The distribution of NPY-IR was highly dense in the supraoptico-hypophyseal zone, but there are many NPY-IR in the spinal trigeminal nucleus, the vagal nucleus, the vague in the solitary tract. Most of the SP-IR were found in the medial and intermedial subdivision of the NTS, fewer in the ventrolateral and very few in the SG. In summary, the coincidence between the distribution of PMT- and NPY-IR is in accord with data obtained from the rat wherein the patterns of the NPY are similar to those of the rhesus monkey.
315.15


315.17

IMMUNOHISTOCHEMICAL STUDIES ON THE VASOACTIVE SUBSTANCES IN THE HYPOTHALAMIC MAGNOCELLULAR NUCLEI. H.Yamada, H.Yamada, Y.Sand. Dept. of Anatomy, Kyoto Pref. Univ. of Med., Kyoto 602, Japan. The distribution of such vasoactive substances as serotonin, vasopressin, endogenous 

digitals-like substances and atrial natriuretic peptide (ANP) in the hypothalamus was studied. Stained the different regions of the hypothalamus with serotonin-, vasopressin- and ANP-antibodies, and then subjected immunohistochemically with ABC method. Few serotonin nerve fibers were seen in the paraventricular nucleus, supraoptic nucleus and its accessory nuclei. In these nuclei, EDLS-containing nerve cell were distributed. Moreover, EDLS was co-localized with vasopressin. However, ANP was observed only in the paraventricular component of the paraventricular nucleus. In the rats fed with high sodium purina chow (4 weeks), the immunoreactivities of vasopressin and ANP were increased while those of digoxin (i.e., EDLS content) were decreased; however, serotonin-immunoreactivity was not changed.

315.16

STAINING OF VASOPRESSIN NEURONS IN ADRENALECTOMIZED RATS USING A VASOPRESSIN-ANTI-IDIOYPE ANTIBODY. G. Bertolino, M. Piaud, and K. Knuut. Neuroendocrine Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 A vasopressin anti-idiotyp antibody was generated by immunization of a rabbit with primary antibody against rat vasopressin neurons in the suprarenal and paraventricular nuclei of the hypothalamus in non-tubulata ren. Staining was reduced or eliminated by preincubation or co-incubation of the antiserum with synthetic vasopressin or rat neural membrane in a dose-dependent manner. The anti-idiotyp antibody inhibited binding of 125I-vasopressin to the neuroendocrine protein in a dose-dependent manner. Preliminary characterization of this antiserum indicates that the anti-idiotyp antibody is a putative vasopressin receptor associated with vasopressinergic neurons following adrenalectomy. It has been established that vasopressin CRF-containing neurons in PVN and hypothalamus are vasopressin neurons and that vasopressin neurons in the paraventricular and magnocellular areas of PVN are vasopressin neurons. We therefore conclude that the putative vasopressin receptor seen by our anti-idiotyp antibody in magnocellular neurons may be present in the paraventricular vasopressin producing neurons of the adenohypophysis.

315.18

DISTRIBUTION OF MORPHOLOGIC AND IMMUNOREACTIVE CELLS IN THE CAT PONTINE PARAVENTRICULAR AREA. M. Ellingsen, F. Orst. Dept. of Physiology, Univ. of Western Ontario, London, Canada N9A SC1. The parabrachial pontine area (PPA) surrounding the brachium conjunctivum of the dorsolateral pontine has been implicated in pain and cardiovascular control mechanisms. In this study the distribution of perikarya and fibers associated with vasopressin-containing neurons in the PPA was examined by using immunohistochemical techniques. Paraventricular neurons in the PPA and its surrounding areas were stained with primary anti-vasopressin IgG. This antiserum immunostains the parvocellular neurons of the paraventricular nucleus containing magnocellular neurons of the PPA and its surrounding areas. Following adrenalectomy it has been established that parvocellular CRF-containing neurons in PVN synthesize vasopressin. We examined the presence of putative vasopressin receptors associated with these parvocellular CRF-vasopressin immunoreactive cells. Rats were adrenalectomized, followed a survival period of 72 hours to 8 weeks, brains were stained with primary vasopressin and the vasopressin anti-idiotyp antibody. The vasopressin anti-idiotyp antibody immunostained neurons in the paraventricular and magnocellular areas of PVN, whereas the anti-idiotyp antibody immunostained magnocellular neurons only. We therefore conclude that the paraventricular vasopressin neurons are not present in the paraventricular vasopressin producing neurons of the adenohypophysis.

LEARNING AND MEMORY: ANATOMY II

316.1

DIRECT PROJECTIONS FROM THE LATERAL PONTINE NUCLEUS TO THE ANTERIOR INTERPOSITUS NUCLEUS: A POTENTIAL CS PATHWAY FOR CLASSICAL CONDITIONING. J.E. Steinmetz and D.R. Sengelaub. Program in Neurobiology, University of California, Southern Calif, Univ Park, Los Angeles, CA 90089-1061. When afferent information is relayed by the olivary complex to the cerebellar anterior interpositus nucleus the pattern of synaptic connections is not clear. The present study was designed to examine the role of the olivary complex in the pattern of synaptic connections. In this study the pattern of synaptic connections of the olivary complex to the cerebellar anterior interpositus nucleus was examined. The results indicate that the pattern of synaptic connections of the olivary complex to the cerebellar anterior interpositus nucleus is not clear.

316.2

PONTINE PROJECTIONS OF COCHLEAR NUCLEI USING ANTEROGRADE HRP OR PHA-L. J.K. Thompson, D.G. Lavond. C. Econopouloos, W. Maurus, and R. Saporito. Dept Psych/CRM 001, Univ Southern Calif, Univ Park, Los Angeles, CA 90089-1061. Previous studies using retrograde fluorescent transport of Fluorogold or of Fast Blue suggest a direct projection from DGN and VCN to posterior aspects of the lateral pontine nucleus (LPN). In other studies, field potentials and multiple unit activity can be evoked in the LPN by auditory click stimul. Both direct electrical stimulation of the LPN as a CS and LPN lesions suggest that this projection may contain auditory information to the cerebellar locus of neuronal plasticity for classical conditioning. The present study was designed to examine whether the cochlear nucleus was anatomically confining the pathway from cochlear nucleus to the LPN by using anterograde tracing techniques. Rabbits were injected with HRP or PHA-L into the cochlear nucleus and the cochlear nuclei were processed and was later examined with both light and dark field microscopy.

Supported by NSP BBS801046A8 and ONR N80001483K0238 to RFT and NINCDS NS2185303 to DGL.
LEARNING AND MEMORY: ANATOMY II

316.3 UNILATERAL INFERIOR OLIVE NMDA LESION LEADS TO UNILATERAL DEFICIT IN ACQUISITION OF NMR CLASSICAL CONDITIONING. M. Mintz, Y. Yun*, D.O. Lavond and R.F. Thompson, Dept. of Psychol., SUN 501, Univ. Southern California, Los Angeles, CA 90089-1061.

A unilateral lesion of the cerebellum permanently abolishes the NMR only to US presented to the eye ipsilateral to the lesion. The control contralateral side of the lesion. This specialization of cerebellar memory sites might be related to the restricted innervation pattern of cerebellar hemispheres as indeed the climbing fibers to each hemisphere convey mainly the US from the ipsilateral eye. To test this hypothesis, we have measured the conditioning deficit to US presented to either of the eyes after denervating one cerebellar hemisphere of its US input. The anteromedial aspect of dorsal accessory olives was localized by antidromic stimulation of cerebellar HVI area and then injected with 40-200 µmol of the neurotoxin NM-DL-A to destroy the cells’ somata while sparing fibers of passage from the contralateral olivary or other fibers as well. Extensive lesions of the IO prevented acquisition of NMR to the denervated cerebellar hemisphere. Conditioning, to the other eye was normal in all rabbits. It appears that specialization of the memory sites in the cerebellum, in respect to the learning situation, originates from the distinctive lack of divergence of sensory input from one eye to the bilateral cerebellar hemispheres.

316.5 Filopodia are present in synaptic glomerulae in the tactile learning area of the cerebellar hemispheres. The climbing fibers to the cerebellar hemispheres contain the usual spherical clear vesicles ±40-nm diameter with prominent neurofilaments and microtubules leading away from the presynaptic endings. The filopodia are present in synaptic glomerulae in the tactile learning region of the cerebellum, in respect to the learning situation, originates from the distinctive lack of divergence of sensory input from one eye to the bilateral cerebellar hemispheres.

316.6 NEURAL SUBSTRATES FOR LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE REACTION VISUALIZED USING 2-DG. REGIONAL CHANGES IN AUDITORY, RETICULAR AND CEREBELLAR SYSTEMS. F. Gonzalez-Lima, T. Finkenstädt* and J.-P. Caumo. Dept. of Anatomy, SUN 501, College Station, TX 77843, and Dept. of Neurotox, Univ. of Kassel, Kassel, F.R.G.

Long-term habituation of the acoustic startle reflex. Group 1 rats were long-term habituated for one week and injected with 2DG on the last session. Group 2 rats were pretrained and then were injected with 2DG and stimulated for one session of short-term habituation. Group 3 rats served as unstimulated controls. Long-term habituated rats showed a significantly greater metabolic activation of the auditory system (with the exclusion of the thalamocortical pathway), the cerebellum (deep nuclei, vermal lobules 1-7, lateral hemispheres) and major cerebellar input-output structures (vestibular nuclei, inferior olive, spinal cord). The largest increase was in the lateral superior olive. In contrast, the midbrain reticular formation and its ascending thalamocortical system (anterior, medial, reticular and lateral posterior nuclei, and the frontal cortex) showed significant suppressions in 2DG uptake. The changes revealed by 2DG are the first demonstration of brain substrates with localized metabolic alterations demonstrating a high correlation between NM, discriminated and raw EMR before and after the lesion. Supported by NINDS 1 R01 NS2185301 to DG and NSF BMS1066648 to RTP.


The locus coeruleus (LC) is believed to play a role in the expression of fear. Both NE -2 agonists and (-2 opioids) are known to have similar effects in suppressing the neuronal activity of LC cells while, corticotropin releasing factor (CRF) is known to enhance the firing of LC cells. This study looked at the effects of (-2 agonists, Clonidine (3 µg) and UK 14,304 (5 µg), a µ opioid agonist, DOLA (10 µg), and the CRF antagonist, Alpha-Helical CRF (9-41) (5 µg) on the development of classically conditioned brazcardia in rats when administered into the rostral fourth ventricle in the vicinity of the LC. All rats were pretrained and then were injected with 2DG and stimulated for one session of short-term habituation. Group 3 rats served as unstimulated controls. Long-term habituated rats showed a significantly greater metabolic activation of the auditory system (with the exclusion of the thalamocortical pathway), the cerebellum (deep nuclei, vermal lobules 1-7, lateral hemispheres) and major cerebellar input-output structures (vestibular nuclei, inferior olive, spinal cord). The largest increase was in the lateral superior olive. In contrast, the midbrain reticular formation and its ascending thalamocortical system (anterior, medial, reticular and lateral posterior nuclei, and the frontal cortex) showed significant suppressions in 2DG uptake. The changes revealed by 2DG are the first demonstration of brain substrates with localized metabolic alterations dependent on long-term habituation.
IBOTENIC ACID LESIONS IN THE MAGNOCELLULAR MEDIAL GENICULATE NUCLEUS PREVENT THE ACQUISITION OF CLASSICALLY CONDITIONED BRADYCARDIA TO SINGLE TONES IN RABBITS. N. Schneiderman, C.S. Markgraf, P.M. McCabe, D.K. Lisowsky, and R.W. Wiseman. Dep. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

Previous work in our laboratory has demonstrated that ibotenic acid lesions in the magnocellular region of the medial geniculate nucleus (mMGN) prevent the acquisition of differentially conditioned bradycardia to acoustic stimuli in rabbits. Although lesioned animals were not able to discriminate between a tone paired with shock (CS+) and a separate tone that was not paired with shock (CS-), the animals did not exhibit a significant bradycardic response to either tone. This suggests that the role of mMGN in conditioned bradycardia to single tones is more significant than the role of mMGN in classically conditioned bradycardia to a single tone.

New Zealand albino rabbits received bilateral lesions via injection of ibotenic acid into either mMGN (m) or into control lesion sites (n=16). Following recovery and habituation to the tone CS (500 Hz, 90dB), the CS was paired with peripheral shock (0.5sec, 3mA) for 30 trials. In half of the control lesioned animals the tone and shock were unpaired in a standard pseudoclassical paradigm (n=8). Control lesioned animals exhibited bradycardiac responses (mean=-11.6 beats/min) that were significantly greater than the pseudoseconded group responses (mean= -0.7 beats/min). In contrast, mMGN lesioned animals showed no significant heart rate conditioning (mean=-4.3 beats/min). These findings suggest that neurons intrinsic to mMGN are involved in classically conditioned heart rate responses to single tones as well as differential conditioning through the more than one tone. These results are consistent with results from studies in other species that have demonstrated that mMGN is critical for the development of cardiovascular and behavioral conditioned responses to aversive stimuli. Supported by NS 24874, HL 27642, and HD 36988.

TRANSSECTION OF THE MIDDLE CEREBELLAR PEDUNCLE ABOLISHES CLASSICALLY CONDITIONED EYELID RESPONSES IN THE RABBIT. R.W. Skelton, Dept. of Psychology, Univ. of Victoria, Victoria, B.C., CANADA, V8W 2Y2.

The middle cerebellar peduncle (MCP) has been shown to be essential to classical conditioning of eyelid responses in rabbits (Lewis et al., 1987). The present study tested the effects of MCP lesions and knife cuts to the MCP on retention of eyelid conditioned responses (CR) in rats.

Male hooded rats were prepared with subcutaneous EMG electrodes for recording CRs and transorbital electrodes for delivery of eyeshock. In addition, lesion electrodes (or guide cannulae) were implanted unilaterally into the MCP (or 1 mm dorsal to it). After recovery from surgery, each rat was trained to a criterion of 80 CRs in a Pavlovian delay paradigm which permitted 450 msec tone CS with a 100 msec eyeshock unconditioned stimulus (2-4 mA, 60 Hz). The MCP was then destroyed bilaterally by passing anodal current through the lesion electrodes or a knife through the guide cannulae.

Transsection of the MCP by either means severely disrupted CRs either startling responses to the tone or unconditioned responses to the eyeshock. In most cases, the cerebellum itself was undamaged. These results confirm and extend previous evidence that the MCP is a critical afferent to the cerebellum for eyelid CRs. (Supported by a grant from NSERC, Canada.)

SPATIAL NAVIGATION: EVIDENCE FOR CEREBELLAR INVOLVEMENT FROM pcd NEUROLOGICAL MUTANTS. K.M. Hamre, C.R. Goodlett, and J.R. West. U. of Iowa, Dept. of Anatomy, Iowa City, IA 52242.

The ability to perform spatial navigation efficiently in the Morris water maze task is known to depend on normal hippocampal function. However, we have found that early postnatal alcohol exposure in rats, which had no significant effect on hippocampal cell numbers, caused severe reductions in cerebellar Purkinje cells, resulted in impairments in spatial navigation. To examine more directly whether Purkinje cell loss may produce spatial navigation deficits, we tested Purkinje Cell Degeneration (pcd) neurological mutant mice, their littermate controls (+/+) and C57BL/6J (B6) mice in the Morris maze. Groups of mice were tested beginning at either 30, 45 or 110 days of age. At all three ages the B6 and +/+ littermate controls performed at a level comparable to the sham and medial lesioned animals. During the second training session the animals were permitted access to a 2.0% vinegar solution and were injected i.p. with either 0.1% LiCl, 1 mg/kg of morpina or their saline vehicle after saccharin removal. The animals with lateral lesions did not form a saccharin aversion, while sham and medial lesioned animals did. During the second tracking session the animals permitted access to a 2.0% vinegar solution and were injected i.p. with either 0.1% LiCl, 1 mg/kg of morpina or their online vehicle after saccharin removal. The animals with lateral lesions did not form as large a conditioned flavour aversion as the sham and medial lesioned animals. Control tests showed that animals with lateral lesions did not taste the flavours. These data indicate that the lateral portions of the parabrachial nuclei are important in the formation or recall of conditioned flavour aversions.
316.15 VISUAL CONCEPT OF FOOD/NON-FOOD IN PIGEONS-EFFECTS ON ECTOSTRIATAL LESION-L. S. Watanabe, Department of Psychology, Keio Univ. Mita 2-15-45, Minato-Ku, Tokyo, Japan.

Two groups of pigeons were trained on food vs. non-food objects. An experimental chamber was an operant chamber with a rectangular transparent pecking key and a conveyor belt was placed. The conveyor belt had 40 small cubicles each contained food or non-food object. One group was taught when the key was foods appeared, and the other group was taught to peck when non-food objects appeared. Generalization to new objects was tested when the birds learned the task. Then the ectostriatum or neostriatum was damaged and the birds were retrained. Generalization was tested again after the relearning.

Both groups could learn the discrimination. They showed generalization to the objects which had not been presented during the discrimination training. After the neostriatal lesion the birds maintained their discrimination, whereas the birds with ectostriatal lesion required longer training to relearn the task. The subjects with damaged neostriatum showed clear generalization after surgery but those with damaged ectostriatum showed weak generalization.

These results suggest that the ectostriatum has an important role in this concept discrimination.

316.16 FOLLOWING BRAIN LESIONS, IF EXPOSED IN D'TEMPS, MEMORY DECAYS AT NORMAL RATES-J. L. Ringo, Department of Physiology, Univ. of Rochester Med. Ctr., Rochester, NY 14642

Data from the literature on the effect of various brain lesions on retention and recognition memory in the monkey was examined. On the basis of percentage scores the published data can be interpreted to signify that the monkey in which recognition is reported can recognize a previously seen objects decays faster in macaques with brain lesions than it does in normal animals.

A re-analysis in terms of the d' of Signal Detection Theory or in terms of an arcsine transform of the percentage values showed, on the contrary, that the rate of "decay" from 0-600 sec is essentially the same in normal animals and in those with lesions. Indeed, the effect of the lesions is fully developed at the shortest times tested, and shows no differential loss as a function of delay between initial presentation and test.

An implication of this result is that the lesion effects are in initial recording or retrieval processes and not in retention or any relatively slow processes following initial presentation.

317.1 CLONING AND EXPRESSION OF MYELIN ASSOCIATED GLYCOPROTEIN, Po-J. Johnson*, M. Tropak* M. Arguint* W. Sadoul, Heidelberg, FRG) and we are currently mapping the neuronal processes (collaboration with M. Schachner and R. Johnson).

MAG variants is developmentally and spatially regulated. We have achieved high levels of recombinant MAG protein expression in transfected mammalian and insect cells. Recombinant MAG protein extracted from transfected NIH/3T3 cells and incorporated into liposomes was found to bind specifically to neuronal processes (collaboration with M. Schachner and R. Sadoul, University of Toronto, Mt. Sinai Hospital Research Institute, Toronto, Ontario). Myelin associated glycoprotein (MAG) is a 100 kD integral membrane protein that is thought to play a role in the neuron-glia interactions that are site specific to the myelinating region.

The extracellular domain of MAG contains five tandem repeats, approximately 90 residues in length, which share homology (20-30%) with the variable and constant domains of immunoglobulins and the neural cell adhesion molecule (N-CAM). The domains are similar to the extracellular domains of the immunoglobulin superfamly and appears to be involved in the interaction of myelin-forming oligodendrocytes and Schwann cells with axons. There is evidence implicating MAG in the pathogenesis of human diseases such as multiple sclerosis and neuropathy associated with GM2 gangliosides. Several laboratories have recently characterized the coding region of the rat brain, but there is evidence for biochemical differences in human MAG which may be relevant to human diseases. Therefore, a CDNA library of the spinal ganglia region of a one-day old infant was screened with a P1-labeled, full-length rat CDNA clone. A positive CDNA was identified and inserted into a plasmid vector. Double stranded sequencing of the 3' ends of the CDNA has identified 83 bases of the non-coding region, a 16 amino acid signal sequence, 122 amino acids of D1, and 45 amino acids of D2. Compared to the rat sequence, there is one amino acid difference in D0, three in D1, and none in the part of D2 so far sequenced. This corresponds to a 98% amino acid sequence homology. The arg-gly-asp cell attachment site in D1 is conserved, and there is an additional potential glycosylation site in D1 of human MAG in comparison to rat MAG.


The myelin-associated glycoprotein (MAG) is an adhesion molecule that is a member of the immunoglobulin superfamly and appears to be involved in the interaction of myelin-forming oligodendrocytes and Schwann cells with axons. There is evidence implicating MAG in the pathogenesis of human diseases such as multiple sclerosis and neuropathy associated with GM2 gangliosides. Several laboratories have recently characterized the coding region of the rat brain, but there is evidence for biochemical differences in human MAG which may be relevant to human diseases. Therefore, a CDNA library of the spinal ganglia region of a one-day old infant was screened with a P1-labeled, full-length rat CDNA clone. A positive CDNA was identified and inserted into a plasmid vector. Double stranded sequencing of the 3' ends of the CDNA has identified 83 bases of the non-coding region, a 16 amino acid signal sequence, 122 amino acids of D1, and 45 amino acids of D2. Compared to the rat sequence, there is one amino acid difference in D0, three in D1, and none in the part of D2 so far sequenced. This corresponds to a 98% amino acid sequence homology. The arg-gly-asp cell attachment site in D1 is conserved, and there is an additional potential glycosylation site in D1 of human MAG in comparison to rat MAG.


Proteoglycans (PGs) are large, complex, glycosylated proteins found in tissues of central and peripheral nervous system (CNS). Myelin, the myelin sheath, is a complex mixture of proteins, lipids, and carbohydrates. It is an important component of the peripheral and central nervous system. The extracellular matrix of the CNS contains a number of proteoglycans that are important in the development and maintenance of the nervous system. These proteoglycans include proteins such as heparan sulfate proteoglycan (HSPG) and chondroitin sulfate proteoglycan (CSPG), which are important in the development and maintenance of the nervous system. The extracellular matrix of the CNS contains a number of proteoglycans that are important in the development and maintenance of the nervous system. These proteoglycans include proteins such as heparan sulfate proteoglycan (HSPG) and chondroitin sulfate proteoglycan (CSPG), which are important in the development and maintenance of the nervous system.

The myelin-associated glycoprotein (MAG) is a 100 kD integral membrane protein that is thought to play a role in the neuron-glia interactions that are site specific to the myelinating region. The domain is similar to the extracellular domains of the immunoglobulin superfamly and appears to be involved in the interaction of myelin-forming oligodendrocytes and Schwann cells with axons. There is evidence implicating MAG in the pathogenesis of human diseases such as multiple sclerosis and neuropathy associated with GM2 gangliosides. Several laboratories have recently characterized the coding region of the rat brain, but there is evidence for biochemical differences in human MAG which may be relevant to human diseases. Therefore, a CDNA library of the spinal ganglia region of a one-day old infant was screened with a P1-labeled, full-length rat CDNA clone. A positive CDNA was identified and inserted into a plasmid vector. Double stranded sequencing of the 3' ends of the CDNA has identified 83 bases of the non-coding region, a 16 amino acid signal sequence, 122 amino acids of D1, and 45 amino acids of D2. Compared to the rat sequence, there is one amino acid difference in D0, three in D1, and none in the part of D2 so far sequenced. This corresponds to a 98% amino acid sequence homology. The arg-gly-asp cell attachment site in D1 is conserved, and there is an additional potential glycosylation site in D1 of human MAG in comparison to rat MAG.


The decrease in Po protein of myelin after axotomy is accompanied by a change in its glycosylation pattern, from a complex to a high-mannose type glycoprotein. It has been suggested that this change results in the diversion of Po to the lysosome where it is degraded. To investigate this, we expressed Po in Chinese hamster ovary (CHO) cells, whereby changes in glycosylation could be directly correlated with changes in protein. A plasmid containing the Po cDNA, with two selectable markers and the Po-cDNA under the control of the CMV promoter, was introduced into two different cell lines. The transfected cells were then selected for expression of Po and Po mRNA. The Po protein was found to be co-localized with the Po mRNA. The Po protein was then immunoprecipitated and analyzed by Western blot analysis. Western blot analysis showed several bands from both cell lines with increased Po protein (2-3 fold). Immunofluorescence studies with both cell types showed a specific pattern resembling plasma membrane staining indicating that Po glycoprotein does reach the plasma membrane. The two cell lines used a detailed assessment of the role of carbohydrates in intracellular sorting of Po protein. (This was supported by Grants NS 21700 and NS 22849.)

These cells are small, round, process-bearing cells which accumulate transferrin (Tf) and express the Tf receptor. 2) Myelin basic protein (MBP) represents the major extrinsic protein of the myelin membrane. It can be fractionated into several charged components. In conclusion, components of MBP are phosphorylated to a different extent and phosphorylation of a single critical site may be influencing the stabilization of the β structure. Inclusion of the critical site is under way.

317.7 PHOSPHORYLATION OF CHARGED ISOMERS OF HUMAN MYELIN BASIC PROTEIN: EFFECT ON SECONDARY STRUCTURE. Supported by NIH grants MS 16945 and HD 04147.

Myelin basic protein (MBP) represents the major extrinsic protein of the myelin sheath. Many of these charged isomers (components) have been analyzed in the cation exchange column. In the present study effect of phosphorylation on the secondary structure of four of these components was investigated by circular dichroism. MBP components were phosphorylated with human brain white matter protein kinase C. The extent of phosphorylation varied considerably from 1 mol of phosphate/mole of MBP in component 1 (C-1) to 6 mol of phosphate/mole of MBP in component 4 (C-4). All non-phosphorylated components exhibited varying amounts of β structure, random coil and turns, but no α helix. Phosphorylation of these components induced changes in secondary structure and caused an increase in β structure to 40-45% for all components. The increase in β structure could not be reversed by removal of 50% of phosphate by acid phosphatase. The other 50% was inaccessible to the enzyme suggesting it occupies a critical site involved in the stabilization of the β structure. Isolation of the critical site is under way.

317.8 IDENTIFICATION OF THE CA++/CALMODULIN-DEPENDENT PROTEIN KINASE IN RAT BRAIN MYELIN FPCTION. Supported by NIH grants NS 05515 and HD 04147.

In conclusion, components of MBP are phosphorylated to a different extent and phosphorylation of a single critical site may be influencing the stabilization of the β structure.

317.10 TRANSFERFERRIN AND ITS RECEPTOR ARE EXPRESSED BY MYELINATING SCHWANN CELLS. Supported by NIH grants NS 22671.

A substance from ground sciatic nerve called “sciatin” was shown to be capable of replacing the requirement for transferrin (TF) in cultures of chicken myoblasts. This substance was later shown to be TF, but the source of the TF in the sciatic nerve has not been determined. In this study, we demonstrate immunohistochemically that TF and the TF receptor are expressed by Schwann cells in the sciatic nerve, but not by those in the thoracic nerve or in the hypotrophic trunk or vago nerve. Following crush of the sciatic nerve TF, the TF receptor and galactocerebroside (GalC) are not detectable in the first day of nerve regeneration by 5 weeks, post-crush some GalC and TF receptor staining is present, but not TF. GalC, TF receptor, and TF are all present by 8 weeks and were analyzed by immunohistochemistry. The abnormal staining pattern continues into the 10th week post-crush. The results of the crush injury study show that the expression of the TF receptor precedes the accumulation of TF by Schwann cells and is at least coincident with the expression of GalC. These data support the general hypothesis that TF accumulation by myelinating cells (oligodendrocytes and Schwann cells) is a necessary condition for the production of myelin. Supported by NIH grants NS 22671.
NEUROGLIA: MYELIN FORMING CELLS

B. Friedman, I.G. Warman-Pacon, E. Friedman, M. Constantine-Paton, S. G. Waxman, Yale Univ. Sch. Med., VA Med. Cntr., West Haven, CT 06516 & Dept. Neurology; study focuses on cells that have not formed myelin but are recognized by oligodendrocytes in cultures free of neurons. Thus our cells may be recognizes mature oligodendrocytes, myelin sheaths and immature stained cells had a range of 48 µm + / — 10 µm (SD) (11 cells, 2 tadpoles). From process from adjacent stained cells mingled and appeared to contact each other. The observed overlap of processes suggests that presumptive oligodendrocytes may interact during early stages of development. Supported by NIH, NMSS & VA.


Monoclonal Antibody OLIGO Recognizes Presumptive Oligodendrocytes Prior to Myelination.

During development a subpopulation of neuronal cells (O-2A progenitors, oligodendrocytes, and type-2 astrocytes) is involved in CNS myelination. To analyze the potential role of this subpopulation in the course of demyelination we examined demyelinating lesions in the spinal cord in 28d old C57Bl/6 mice by intracerebral injection of MHV-A59 coronavirus. At 4-5 wks post-infection (p.i.) vacuoles and demyelinated axons were seen in the sciatic nerve. Remyelination was already initiated and resulted in extensive myelin repair in the following 8-12 wks. Neuronal cells were isolated from spinal cord by combinate enzymatic and mechanical dissociation along with Percoll gradient centrifugation, cultured for 1d, and stained simultaneously for O4 antigen, galactocerebroside (GC), and glial fibrillary acidic protein (GFAP) with 3-color immunofluorescence and confocal microscopy analyses. The number of O4, GC, and GFAP expressing cells continued to increase in vivo with 3H-thymidine could be isolated from lesioned but non control adult spinal cord. These findings may be explained by gliogenesis and/or phenotypic changes of O-2A lineage cells which may occur in the origin of neurofilaments and during demyelinating diseases. Cultured Schwann cells were prepared from 3d rat sciatic nerve. Schwann cells were treated with histamine or the MEF. By autoradiography, 2mM histamine alone did not increase the number of labelled cells. The addition of 2mM histamine to MEF treated Schwann cells resulted in a 50% increase in labelled cells. In contrast, the addition of histamine did not alter the mitogenicity of the AEF. Thus, histamine specifically enhances Schwann cell proliferation induced by MEF, and may play a role in cellular proliferation occurring in certain disease states. Supported by Coqlate Research Council.


In vitro analysis of OLIGO and/or phenotypic changes of O-2A lineage cells which may occur in the origin of neurofilaments and during demyelinating diseases. Cultured Schwann cells were prepared from 3d rat sciatic nerve. Schwann cells were treated with histamine or the MEF. By autoradiography, 2mM histamine alone did not increase the number of labelled cells. The addition of 2mM histamine to MEF treated Schwann cells resulted in a 50% increase in labelled cells. In contrast, the addition of histamine did not alter the mitogenicity of the AEF. Thus, histamine specifically enhances Schwann cell proliferation induced by MEF, and may play a role in cellular proliferation occurring in certain disease states. Supported by Coqlate Research Council.

DEVELOPMENTAL REGULATION OF CHANNEL EXPRESSION IN OLIGODENDROCYTES. G. H. Sheepman, A. J. Moond, H. Schechner and H. Kettlenmann (SPON: C. ten Bruggencate) Dept. of Neurobiology, Univ. Heidelberg, INF 364, 6900 Heidelberg, FRG. We characterized membrane currents in cultured brain derived murine oligodendrocytes at different developmental stages, using the patch-clamp technique. Recordings were made from O-2A progenitor cells which could be identified as such by their reaction to the monoclonal antibody OLIGO (Weiner & Osserman, 1984, J Cell Biol. 99:3290). The addition of ammonium chloride, phorbol esters, calcium chelators, or lithium ions modulates the kinetics of the voltage-sensitive sodium channel. The activation and inactivation voltages of the sodium channel are shifted toward hyperpolarizing potentials in the presence of the MEF. By autoradiography, 2mM histamine alone did not increase the number of labelled cells. The addition of 2mM histamine to MEF treated Schwann cells resulted in a 50% increase in labelled cells. In contrast, the addition of histamine did not alter the mitogenicity of the AEF. Thus, histamine specifically enhances Schwann cell proliferation induced by MEF, and may play a role in cellular proliferation occurring in certain disease states. Supported by Coqlate Research Council.

MELANIN PROTEIN BIOSYNTHESIS BY CYSTICAL SYMPTOMATIC SCHWANN CELLS. Brian M. Gooden and Jan E. Yoshimura, Dept. of Neurosciences, Dept. Psychology, Colgate Univ, Hamilton, NY. The expression of myelin antigens, GFAP, and transferrin in the developing rat optic nerve. J. W. Connor and M. H. Litw. (SPON: R. Bohn). Dept. of Anatomy, H.S. Harshey Medical Center, Hershey, PA 17033. Extrinsic factors (e.g., neuronal activity) appear to exist which influence or possibly control the process of myelination. Recently, the iron transport protein, transferrin (TF), has been identified as a regulator of myelination suggesting that iron and TF could play a role in myelogenesis. In the present study, the developing rat optic nerve was examined immunohistochemically for the presence of TF in basic protein; MBP and the transferrin receptor; Tfr, galactocerebroside, Tf, and glial fibrillary acidic protein. Tfr and Tf were positive in the outer limiting membrane of Schwann cells at PND 8. Patches of MBP and GaIC positive fibers were also present. The number of Tfr positive cells peaked between PND 7-15 and were generally perivascular. The number of Tfr, GaIC and MBP cells continued to increase into adulthood and were found throughout the optic nerve. These results demonstrate that Tfr positive oligodendrocytes appear at the time myelin begins and are most numerous during the highest rate of myelination and then decrease thereafter. Furthermore, the presence of TF in oligodendrocytes precedes the expression of MBP and GaIC in oligodendrocytes. These data are further support of the hypothesis that TF expression by oligodendrocytes is a necessary condition for the process of myelination. This work is supported by Grant NS-22671.
317.17 MYELIN DEGRADATION IN SCATIC NERVE EXPLANTS. T. W. Reynolds, K. P. Coates, and H. C. Kallman. Department of Anatomy, The Pennsylvania State University, College of Medicine, Hershey, PA 17033. Treatment of rat brain synaptic membranes with the non-competitive glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in hippocampal slices (in 1 mM Mg). CNQX (1-30 μM) produced a parallel shift in the log10 response curves and gave the following KD values (μM); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 2; kainate, 2; N-methyl-D-aspartate (NMDA), >30. Low frequency (0.033 Hz) synaptic responses, elicited by stimulation of Schaffer collateral commissural fibres, were blocked by 10 μM CNQX. However, with high intensity or high frequency (100 Hz, 0.5-1 s) stimulation a component of AMPA responses which was reversibly blocked by a selective NMDA antagonist.

317.18 THE DEVELOPMENT OF OLIGODENDROGLIUM IN CULTURE. R. E. Podolsky, K. Miller, and C. F. Pak. Johns Hopkins Univ. Sch. of Med., Dept. Neurology, Baltimore, MD 21205. Oligodendroglia elaborate extensive quantities of membrane which endows axons to form the multimellar highly compacted myelin sheath. It is possible to obtain oligodendroglia by either differential plating methods or by synthesizing a synthetic substrate. The differential plating method involves primary cultures prepared from neonatal rat brain. The bulk-isolation technique uses brain tissue from actively myelinating adolescent rat or dissected white matter from bovine brain. The rat oligodendroglia elaborate extensive networks of processes in culture but produce no myelina. Bovine oligodendroglia in suspension cultures produce whorls of membrane which biochemically appear to be early forms of myelina. Thes rat the rat oligodendroglia appear to be less differentiated than bovine ceils. The different stages of oligodendroglia were analyzed for the synthesis of specific fibrils in the presence of various agents known to affect cell differentiation. In this study, evidence of further development in culture would be increased synthesis of cerebroside, a myelin component. Retinoic acid and thyroid hormones appeared to have a positive effect on cerebroside synthesis, while 5-azacytidine and phorbol esters had a negative effect. Retinoic acid had a slight stimulatory effect. Other agents such as sodium butyrate had no effect. (Supported by grants from NIMH NS 14577 and HD 16567).

317.19 CHANGES IN CNS MYELIN FORMATION IN RESPONSE TO A REDUCTION IN OLIGODENDROGLIA. T. J. Sims and S. A. Gilmore. Department of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205. Exposure of lumbarsal spinal cords of 3-day-old rats to ionizing radiation induces a marked reduction in gilla. Although myelin formation is delayed in both dorsal and ventral funiculi, axons remain healthy and increase in diameter. Reconstitution of the gilla population occurs with time but differs between these funiculi. Myelin formation by oligodendrocytes in the dorsal funiculi occurs during oligodendrocytes are responsible for this delayed myelination ventrally. Differences in the pattern of oligodendrocyte myelination are observed more frequently in the DF where the oligodendrocytes are less differentiated than bovine cells. The different stages of oligodendrocyte myelination are observed more frequently in the DF where the oligodendrocytes are less differentiated than bovine cells. The different stages of oligodendrocyte myelination are observed more frequently in the DF where the oligodendrocytes are less differentiated than bovine cells.

317.20 GENERATION OF CYTOTOXIC LYMPHOCYTES FOR HUMAN GLIAL CELLS IN CULTURE. T. C. King, J. C. Calne, F. R. MacLennan, and J. P. Antel. Montreal Neurological Institute, McGill Univ., Montreal, Quebec, Canada H3A 2B4. Cellular immune mechanisms directed against the myelin forming oligodendrocyte (OCy) could contribute to tissue injury in human demyelinating disease. We attempted to measure susceptibility of OCy to cell-mediated cytotoxicity using OCy enriched cultures derived from surgically resected tissue in a 1^1Cr release assay. In a lectin-dependent, non-MHC restricted system, Concanavalin A stimulated lymphocytes were cytotoxic for glial cells (mean specific lysis 30% in 4:1 ratio). Allogeneic CBY lymphocytes activated in a mixed lymphocyte reaction against glial cell donor's lymphocytes also induced specific cytolyis of the donor's glial cells. The CBY subset comprises MHC class I restricted antigen specific cytotoxic T lymphocytes, as well as non-MHC restricted natural killer (NK) cells. OCy were not lysed by non-activated lymphocytes, the usual means to assay for NK cell activity. Immunohistochemical studies indicated that the OCy (GalC^+ cells) express MHC class I but not class II antigens in vitro.

318.1 ION FLUX AND PATCH CLAMP MEASUREMENTS OF PARTIALLY PURIFIED GLUTAMATE-ACTIVATED ION CHANNELS. E. K. Michaelis, A. L. LeR, J. A. Ueda, and T. Kishino. Department of Pharmacology and Center for BioMed. and Bioanalyt. Res., Univ. of Kansas, Lawrence, KS 66046. Treatment of rat brain synaptic membranes with the non-competitive glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in hippocampal slices (in 1 mM Mg). CNQX (1-30 μM) produced a parallel shift in the log10 response curves and gave the following KD values (μM): α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 2; kainate, 2; N-methyl-D-aspartate (NMDA), >30. Low frequency (0.033 Hz) synaptic responses, elicited by stimulation of Schaffer collateral commissural fibres, were blocked by 10 μM CNQX. However, with high intensity or high frequency (100 Hz, 0.5-1 s) stimulation a component of AMPA responses which was reversibly blocked by a selective NMDA antagonist.

Field EPSPs were evoked by stimulation of two independent sets of convergent fibres. Long-term potentiation was induced specifically in one set and the stimulus intensity adjusted so that both pathways elicited EUFS of a similar size. CNQX blocked both the potentiated and the non-potentiated response in parallel. We conclude (i) NMDA receptors can be sympathically activated after blockage of non-NMDA type receptors and (ii) the same type of CNQX-sensitive receptor is likely to mediate both potentiated and non-potentiated responses. CNQX was generously provided by Dr. T. Honoré (Ferrosan).
318.3 Mg**, EXCITATORY AMINO ACIDS, AND DORSAL ROOT POTENTIALS IN THE FROG. J.C. Heckenlively and R.A. Davidoff. Neurophysiology Lab., VAMC and Dept. of Neurology, Univ. of Miami School of Med., Miami, FL 33101. Mg** inhibited the voltage-sensitive tail current in the in vitro frog spinal cord is unexplained. We investigated the role of Mg** since most frog Ringer’s solutions omit Mg** although frog DRPs contains Mg**.

318.4 COMPARISON OF CURRENTS PRODUCED BY ELECTRODECUT UPTAKE OF L-GLUTAMATE AND L-ASPARTATE IN GLIAL CELLS ISOLATED FROM TIGER SALAMANDER RETIN. H. Brew (SPONSORED BY MAIL) Dept. of Physiology, University of London, London, U.K. L-glutamate evokes an inward current in whole-cell clamped retinal glial (Müller) cells, due to the activation of a non-NMDA receptor (AMP-A) glutamate uptake system (Brew & Attwell, 1987). We tested the isolated Müller cells in holding current of -54 mV, 10-30 sec, using the whole-cell patch-clamp technique. We have previously shown that the 10-30 sec synaptic response was blocked with 10 µM APV, which is a non-NMDA receptor antagonist. Thus, we used 100 µM APV to test the whole-cell clamped Müller cells. The 10-30 sec synaptic response was blocked in the presence of 10 µM APV. In the absence of APV, the 10-30 sec synaptic response was not blocked.

318.5 INTRACELLULAR RECORDINGS OF A SLOW NMDA RECEPTOR-MEDIATED EPSP IN DEVELOPMENTAL STAGE AMPHIBIAN SODAL INTRINSIC CIRCUITS. K. Matsuyama and Y. Wada. Institute for Neurosciences, National Institute for Physiological Sciences, Okazaki, Japan. Intracellular recordings were made from the developing retinal ganglion cells of the salamander, Ambystoma tigrinum. The records were obtained from axons, dendrites, and somata of the retinal ganglion cells. In the presence of 2 mM APV, the slow NMDA-mediated EPSP was reduced to less than 10% of the control level. The slow NMDA-mediated EPSP was mediated by a non-NMDA receptor.

318.6 TWO DISTINCT QUISQUALATE RECEPTORS IN ISOLATED RETINAL INHIBITORY AND EXCITATORY CELLS OF RODents. E. Aizenman, A. Karschin*, and S.A. Lipton. Div. of Neuroscience, State Univ. of New York Health Science Center, Buffalo, NY 14215, and Max-Planck Inst für Hirnforschung, D-6000 Frankfort, FRG. We have examined the role of quisqualate in the inhibition of rat retinal ganglion cells. In the presence of 10 µM APV, quisqualate produced a non-NMDA-mediated EPSP in the inhibitory cells. In the presence of 10 µM APV, quisqualate produced a slow NMDA-mediated EPSP in the excitatory cells.

EXTRACELLULAR CALCIUM PROMOTES DESENSITIZATION OF THE NMDA RECEPTOR IN THE MATURE CEREBELLAR PURKINJE NEURON IN VITRO. Andrea J. Yool and Donna L. Gruol, Division of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA.

Subsets of the NMDA receptor have been identified by three agonists, quisqualate (QA), kainate (KA) and N-methyl-D-aspartate (NMDA). Using whole-cell patch clamp, we have characterized the responses to agonists in the mature cerebellar Purkinje neuron (PN) in vitro. The PN is insensitive to NMDA; QA is the most potent agonist. QA (0.5-2 µM), applied as a brief pulse by pressure pipette, elicited a long voltage-dependent response which shows no further desensitisation with repeated applications of QA (0.5-2 µM). The plateau may be mediated by cationic channels permeant to both Na+ and Ca2+. Voltage-dependence for QA was evaluated using voltage-clamp, after latching solenoid valves and a peristaltic pump. The solution exchange rate was 10 µM diameter, was mounted on a stepping motor driven manipulator, and fed via latching solenoid valves and a peristaltic pump. The solution exchange rate was about 20 ms, and produced rapid responses to agonists.

This system was used to apply excitatory amino acids to cultured hippocampal neurons under voltage clamp conditions. Quisqualic acid produced an initial inward current response which decayed rapidly to a stable plateau. With 5 µM quisqualate the time constant of desensitisation was 72 ± 20 ms (n = 5), and the ratio peak/plateau current 1.80 ± 0.82. The initial fast response to quisqualate recovers slowly following desensitisation, and subsequent applications of quisqualate activate only the plateau current, which shows no further desensitisation with repeated applications of agonist. The rapid nature of the initial response to quisqualate hinders study with conventional techniques, and in our previous experiments application of quisqualate from puffer pipettes frequently evoked only the plateau response.

Preliminary cultures with 20 µM concanavalin-A for 10 minutes, or application of concanavalin-A to single neurones from which control responses to quisqualate had been recorded, produced an irreversible block of desensitisation. Experiments are in progress to examine the effects of lectins on responses to other excitatory amino acids.

We thank Jon Johnson for advice on rapid perfusion techniques.

To study the pharmacology of excitatory amino acids on CA1 hippocampal pyramidal cells, a new method was developed using silico-his that included only area CA1 and the stratum radiatum. Longitudinal slices of 635-micron thickness were cut from the caudal hippocampal formation of the rat and the region inferior and fascia dentata were dissected away. Each slice was then transferred to a 2-compartment superfusion chamber, and pyramidal cell bodies and dendrites in area CA1 were isolated from their synapses in the subiculum by using a gas-burette. 2-compartment slices were then treated with each excitant at 35 °C and depolarizing responses of pyramidal cells were recorded relative to their axons in the subiculum. NMDA, AMPA, kainate and L-glutamate depolarized CA1 pyramidal cells. Tetrodotoxin also blocked the responses to these excitants. EPCq values in the absence of Mg2+ were 4.7, 6.9, 9.4 and 1800 uM, respectively. 2-MPA also reversibly reduced responses to AMPA. It appeared to be about 10 times more potent in the reverse micelles in response to NMDA, but it reduced responses to AMPA by a maximum of about one-third. NMDA receptor antagonist 2-MPA significantly altered responses to AMPA.

These results suggest the presence of a substantial NMDA receptor reserve CA1 pyramidal cells. In addition, the activation by AMPA of the NMDA-sensitive high conductance state of the quisqualate receptor-channel appears to account for a substantial portion of its depolarizing action in this system. (Supported by NIH grant NS 16064.)


Electrical stimulation of parallel fibers causes an extracellular alkaline shift (AS) in the molecular layer (Krag et al. J. Neurophysiol 49:851-30), which is blocked by kynurenic (Cheles et al. Soc. Neurosci). We have developed the AS caused by iontophoresis of aspartate (Asp) in the n-inu-turbo cerebellum (Pseudemys scripta elegans). A pH-sensitive microelectrode and iontophoresis were used to detect AS. Complete depolarization was avoided with tips 60-90 µm apart. With superfusion of 40 mM HCO3- buffered Ringer, iontophoresis evoked an AS of 0.04 pH in the ML. Mn2+ (3-5 mM) abolished the late component of the peduncular-evoked field potential (indicating that synaptic transmission was blocked) and reduced the iontophoretically-evoked AS in the GCL by 50%. The predominance of the Asp-evoked AS in the GCL is consistent with the distribution of similarly depolarized CA1 pyramidal cells which in rat are most densely distributed in the GCL (Greenamyre et al. J. Physiol. 351:233-244, 1985). (NS-10164 & NS-07745)

318.18 GLUTAMATE AUTORECEPTORS REDUCE EPC's IN CULTURED HIPPOCAMPAL PYRAMIDAL CELLS. I.D. Forsythe and J.D. Clements. Lab. of Neurosurgery, Developmental Neurobiology, NICHD, NIH, Bethesda MD 20892.

Recordings were made from pairs of mouse hippocampal neurons grown to confluence in plastic petri dishes in a 2-compartment superfusion chamber. The post synaptic cell was voltage clamped and depolarized with current pulses, while the axon of the pre-synaptic cell was recorded. A small depolarization was found to be elicited by the release of glutamate from the axon terminals. The depolarization showed that both substances acted at a presynaptic site to reduce glutamate release. These observations strongly suggest that a fourth EAA receptor, which has a high affinity for glutamate and is distinct from kainate, quisqualate or NMDA receptors, functions as an autoreceptor for another synaptic pathway.

318.19 NOVEL RECOGNITION SITE FOR L-QUISQUALATE SENSITIZES NEURONS TO L-2-AMINO-4-PHOSPHOBUTYRATE. Edward R. Whittemore and James F. Koerner. Dept. of Biochemistry and Neurosciences, Grad. Program, Univ. of Minn., Minneapolis, MN 55455.

Brief exposure of rat hippocampal slices to L-2-amino-4-phosphobutyrate (L-DAP) (30 µM) mimicked the action of glutamate. Neurons were exposed to L-DAP and killed after inward current at these concentrations. Statistical analysis of the fluctuations in epsp amplitude showed that both substances acted at a presynaptic site to reduce the probability of transmitter release. These observations strongly suggest that a fourth EAA receptor, which has a high affinity for glutamate and is distinct from kainate, quisqualate or NMDA receptors, functions as an autoreceptor for another synaptic pathway.

318.20 THE NMDA ACTIVATED CURRENT IN HIPPOCAMPAL NEURONS IS HIGHLY SENSITIVE TO L-GLUTAMATE AND GLUTAMIC ACID AMIDE. Martin Norris, Marc Dichter and Cha-Min Tang. (SPONS: E. Kisch.) Dept. of Physiology and Neuropharmacology, U of Pa., Philadelphia, PA.

The interactions of [H+] on the channel behavior are complex. In general acidification to pH 6.5-7.0 leads to only small suppression of the NMDA activated current, however, shows a greater sensitivity to external pH. In superior collicular neurons (Grant & Lux) (NEuro Sci Let, in press) the trans- component of the NMDA activated current is highly sensitive to [H+]. We found that in rat hippocampal pyramidal neurons both the transient and the persistent components of the NMDA activated current were highly sensitive to acidification. Glutamate, kainic acid and glutimide did not demonstrate this degree of [H+] sensitivity. (Supported by NIH NS 17944.)
Adenosine has prominent neuromodulatory actions in the hippocampus. We have recently demonstrated in the rat hippocampus that adenosine activates protein kinase C (PKC) blocks the effects of adenosine on synaptic transmission in the dentate gyrus. Therefore, PKC activation in low Ca^2+ long-term potentiation (LTP) following n-methyl-d-aspartate (NMDA) receptor activation, we assessed whether NMDA receptor activation also blocks the action of adenosine. Application of adenosine inhibits the extracellularly recorded population spike (PS). Removal of magnesium from the perfusion buffer for 30 minutes results in a subsequent block of adenosine inhibition of the PS. This effect persists after re-introduction of magnesium to the perfusion buffer and is due to activation of NMDA receptors, as it is blocked by 20 μM di-2-amino-5-phosphonovalerate (APV) and does not occur if the perfusion path is not stimulated during the magnesium-free period. APV does not block the action of phorbol esters. Thus, activation of NMDA receptors in the dentate gyrus can be induced in adenosine action, which may be mediated via PKC activation.


Glutamate can activate receptor-coupled ion channels; however, little is known about the ability of this neurotransmitter to modulate voltage-gated ion channels. We have now observed, using isolated dorsal root ganglion (DRG) neurons, that glutamate not only activated an inward current in these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. The Ca(t) currents were isolated from DRGs using tetrodotoxin and cell-attachment. Neurons thus isolated consisted of soma without extensive processes, making them suitable for whole-cell recording. Ca(t), studied under voltage clamp conditions in media which isolated Ca(t) currents, was elicited by brief jumps to a membrane potential of -35mV from a -80mV holding potential. Glutamate inhibition of Ca(t) was independent of the high-threshold, sustained Ca^2+ current (5% inhibition by 1mM glutamate), elicited by brief jumps to 0mV from a -50mV holding potential. In current clamp experiments glutamate, kainate or NMDA produced by Ca(t) (G. White et al. this meeting). Since synapses onto these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. 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Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively selective inhibition of a low-threshold transient (Ca(t)) current. Neurons were isolated from these cells, but also induced a relatively sel...
REFLEX FUNCTION: GENERAL


Two types of background plasticity of the startle reflex have been examined in rats.

PREPULSE-INHIBITION: Startle was elicited by 10 kHz tone and broadband noise at 135 dB SPL. When a short prepulse (2.5-48 kts, 68 dB SPL) was given - which itself does not elicit a startle response - it increased the amplitude to suprathreshold stimuli decreased. The prepulse inhibition in these experiments is the more effective the more broadband the stimulus. Thus, the higher the activation of the auditory system, the more effective the prepulse inhibition.

BACKGROUND NOISE MODULATION: Amplitude and threshold of startle can be modulated by background noise. Narrow band noise (bandwidth 2 kHz, 65 dB SPL) with the center frequency being the stimulus frequency (~3 kHz) had a masking effect. Noise with the center frequency well below (~7 octaves) the stimulus frequency increased the startle response with little effect on the startle threshold. Noise with the center frequency well above (~1.7 octaves) the stimulus frequency resulted in a decrease of the startle response and an increase of the startle threshold. The high frequency noise inhibition can not be explained by simple masking, while the low frequency noise facilitation seems to work by an enhancement of the state of the 'arousal' system. Supp. by NRC SFS 397.

STRICHRINE ENHANCES THE FACILITATION OF THE SPINAL MONOSYNAPTIC REFLEX BY A GLYCINE ANTAGONIST. B.D. Goldstein and N.A. Ibrahim. Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Conditioning volleys in sural (SO) and medial gastrocnemius (MG) nerves were found to produce a biphasic effect on the monosynaptic reflex (MSR) evoked from the MG. An initial facilitation (FAC) of 20% and 60% above the control which peaked at 3 and 2 sec was followed by an inhibition (INH) of 32% and 74% below the control which peaked at 15 and 20 sec after conditioning in the SU or MG nerves, respectively. Pharmacological properties of the FAC and the INH were studied by i.v. injection of physostigmine (PHY) or a ganglion blocking agent. Recent data generated in this laboratory (Ibrahim and Goldstein, Neurosci. Abs. 13:1064, 1987) have demonstrated that physostigmine (PHY) modifies the monosynaptic reflex (MSR) evoked in the L7 dorsal root of spinal motoneurons in a dose and time dependent manner. The low dose of PHY (0.8 mg/kg, i.v.) produced a facilitation lasting for three hours, whereas, the high dose (2.0 mg/kg, i.v.) was found to produce an initial depression which peaked at five minutes followed by a facilitation which was maintained for three hours after the injection. PHY is a blocker of NMDA and spinal fixation prompted us to examine the effects of PHY on spinal fixation, on spinal flexion.

Electrical stimulation to a hindlimb produces a maintained flexion which persists for several minutes. This flexion is caused by the spinal cord ("spinal fixation"). NMDA receptors are found in the spinal cord and have been implicated in long term potentiation (LTP). Simularities between LTP and spinal fixation prompted us to examine the effects of PHY on NMDA receptor, on spinal fixation. Under anesthesia, electrical stimulation (1.5-2 mA, 7 msec, 100 Hz for 60 sec) of a rat hindlimb produced a flexion which was measured by applying 0.5 g weights until stimulated and contralateral legs were equal. At 72 h, rats were anesthetized and remeasured. Then the spinal cord was transected at T7 and flexion measured.

In control rats, flexion following stimulation was 24.4 g. At 72 h, controls retained 14.8 g. Our prior work suggests that this increase reflects the removal of descending serotonergic inhibition. Experimental rats received the NMDA antagonist, MK-801 (10 mg/kg, i.p.) 1 hr prior to stimulation. In these rats, flexion immediately following stimulation was 15.6 g. At 72 h, MK-801 treated rats retained 10.8 g and after transection, flexion hindlimb = 9.0 g. These results indicate that NMDA receptors are involved in the development of spinal fixation and in the circuitry affected by descending serotonergic inhibition.
SPATIAL FACILITATION OF CUTANEOUS REFLEX PATHWAYS TO TRICEPS SURAE IN UNLIMBED AND CHRONIC SPINAL CATS. Labella L A and McCrea D, Dept. Physiology, Univ. Man., Wpg, CANADA R3E 0W3.

We previously found that excitation from the caudal sural nerve predominates specifically in MG motoneurons while inhibition predominates in LG and SOL. On the other hand, effects from the lateral sural nerve was obtained in this study to determine whether the excitatory sural pathways may be disynaptic (see also Baker et al, Brain Res 420, 1987). If this is true, spatial and temporal facilitation in sural reflex pathways may be altered after chronic spinalization. Further, if the apparently special excitatory and general inhibitory sural pathways to TS are under different patterns of descending control, one might predict differential changes in peripheral convergence following chronic cord transection. Experiments to test these ideas are in progress. Supported by the MRC of Canada.

MUSCLE AFFERENTS IN THE ANESTHETIZED CAT. L. Hayward. U. Wesselmann and W.Z. Rymer, Dept. of Physiology, Northwestern University, Chicago, IL 60611.

Recent evidence indicates that feedback from a fatigue-muscle complex contributes to the decline in motor unit firing rate which occurs during fatigue-voluntary contractions (Bigland-Ritchie, B., et al., Physiol. (1984). It has been hypothesized that small diameter muscle afferents are responsible for mediating this fatigue-related reflex since these afferents are sensitive to thermal and metabolic changes in muscle (Brown & Meyer, J. Physiol., 378: 363, 1986). The role of these afferents in this fatigue-related reflex was evaluated by comparing the response characteristics of group III and IV muscle afferents before and during muscle fatigue. Small diameter, slow conducting (1-2 m/s) muscle afferents in the triceps surae were isolated and recorded from L7 and S1 dorsal roots in barbiturate anesthetized cats. Muscle force, length and afferent firing rate were recorded and stored on computer during muscle stretch, twitch, tonic contraction and surface manipulation, before and during muscle fatigue. Preliminary results from 10 afferents demonstrated that muscle fatigue does induce significant increases in group III discharge rate (paired Student's t-test, p<0.05). Three afferents showed significant increases in spontaneous firing rate (118-1000%) and 3 other afferents showed significant increases in discharge rate during muscle stretch and surface pressure that were not statistically significant. There were no reductions in afferent discharge. The time course of recovery of one group III afferent (vol 28.0m/s) that began discharging spontaneously during fatigue was followed for 3 minutes during which the afferent's spontaneous firing rate gradually declined until it was silent. This time course of recovery is similar to the time reported for human motor unit firing rate to recover following fatiguing maximum voluntary contractions. These preliminary data suggest that muscle fatigue does increase the small diameter muscle afferent response, supporting their hypothesized role as fatigue-sensitive muscle afferents.


Chronic spinal hemisection leads to spasticity (Carter et al, Soc Neurosci Abstr 12: 1422, 1986) and to enhanced ventral root reflexes (Bidd, Hultborn, et al., Exp Brain Res. 60: 425, 1986). We tested muscle-specific reflexes using mechanical inputs and outputs in acute decerebrate cats, 11 mo following chronic low lumbar spinal hemisection. Reflexes associated with soleus (SOL), gastrocnemius (G) and tibialis anterior (TA) were measured and compared with those of control intact animals (Munsoh et al, Soc Neurosci Abstr 12: 1422, 1986). Muscle weights were normal but slightly smaller on the lesioned side. Autogenous reflexes were larger on the lesioned side, but not consistently. Inhibition from TA to SOL was observed on both sides in control animals, but was larger on the lesioned side. When stimulation from S1 to TA was weak or absent in both control and lesions. The forced-dependent inhibition from G to SOL was present also on both sides, but the force threshold was lower on the lesioned side. These data demonstrate that the patterns of reflex interactions following lumbar spinal hemisection are similar to those of control preparations. The principal differences from normal, both on the lesioned side, are the reduced force threshold for G to SOL force-dependent inhibition, and the SOL inhibition (Supported by NS15913 (JBM), NS26883 (LAR), MRS of VA (GWS) & NS20655 (THN)).
REFLEX FUNCTION: GENERAL

COMMISSUROTOMY IMPAIRS BIHEMISPHERIC COORDINATION OF abnormal. In at least one of the monkeys the deficit was then transected. The surgery slowed pursuit for a model of spindle response whose output was proportional to the low power of stretch recruitment of new units improved linearity during the later phase of stretch. Step increase in motoneuronal discharge of already recruited units prevented yield to some extent but responses during the later phase of stretch were less linear. Based on these and Lax’ s modification, have shown that yield can be prevented by recruiting new motor units, whereas the stiffness of areflexive electrically activated muscle falls abruptly (a condition called ‘yield’) after the amplitude of stretch exceeds a fraction of a millimeter. The patterns of responses and the subsequent increase in motoneuronal activity, manifested as increase in EMO, strongly suggest that these reflex components are primarily responsible for linearization of muscle output. However, the fact that the reflex components were minimal before or near the yield (depending of stretch velocity) indicates that some form of predictive mechanism is involved. In this study, we modeled the behavior of muscle under a variety of activation conditions. Computer simulations of muscle response, based on Huxley’s model with Zahu- laski’s modification, have shown that yield can be prevented by recruiting new motor units in order of increasing size, starting shortly after the onset of stretch. Subsequent recruitment of new units improved linearity during the later phase of stretch. Step increase in monosynaptic discharge of already recruited units prevented yield to some extent, but responses during the later phase of stretch were less linear. Based on these and previous results obtained during electrical stimulation of cat triceps surae muscles (which suggested that the yield is more effectively prevented by recruiting more motor units after the onset of stretch), we produced a model of monosynaptic pool. This model consisted of a group of threshold devices, each representing a group of motoneurons with similar firing properties. The resulting recruitment level of those devices is stationary. Two sets of scans for each target motion (left), constant velocity to the left; and constant velocity to the left, were obtained during a single session from each subject (47), the control scans were subtracted from each of the activated scans, and the resulting difference images were averaged for each of the stimulation conditions. The cortical areas active during pursuit eye movements include a dorsal area in parieto-occipital cortex (PO) and a region including primary and adjacent visual areas, the supplementary motor cortex. Horizontal saccades elicit responses in PO, the region for eye movements, and the primary and secondary visual areas, the supplementary motor area, cerebellum and the frontal eye fields. No responses were seen in T0. Area T0 is a post-synaptic processing region that may be homologous to area MT in macaques (ARVO abstract, 1988). Since T0 is activated during pursuit eye movements and is near the MT homologue, TO may be homologous to area MST, a region in macaque cortex active during pursuit eye movements.
320.3


Do patterns of connectivity of the supplementary eye field (SEF) more closely resemble those of the arcuate frontal eye field (FEF) or other cortical motor regions? VCA-HRP and fan-lobe histochromatographic methods were used to study pathways of the SEF, FEF, SMA and primary motor cortices. In 2 monkeys the SEF was defined via recording and stimulation methods.

Only SEF and FEF injections labeled pre-oculomotor nuclei. SEF as well as FEF project to the ipsilateral pretectum (n. latens), optic tract and interstitial nuclei of Cajal. All injections labeled the superior colliculus. SEF projects to layers II, III and IV and FEF to I-IV. SMA projects to areas II and IV to VI. Specific pathways were found from SEF to the raphe magnus region and from FEF to n. olivaris pretectalis.

Areas injected posterior to the terminal nucleus varied according to the locus of the injection (e.g. FEF alone to n. dorsolateralis). The pontine tegmentum exhibited dense anterograde label in a reticularis tegmentis pontis; dorsal injections labeled the medial, and lateral injections the lateral region of this nucleus.

(Supported by USPHS Grants EY05879 and EY02305).

320.5


The striate cortex provides the major input to motion processing areas of extrastriate cortex in primates. A minor input to extrastriate cortex may also be provided directly from subcortical structures. The minor input might be evident as a weak capacity for motion processing after loss of striate cortex.

We tested motion processing after loss of striate cortex in 2 patients, following bilateral lobectomies 3 and 10 years before entry. Tissue loss was documented by CT and was evident behaviorally as an absolute hemianopia. Motion processing was measured as transient pursuit eye movements evoked in 750-1500 individual trials of ramp stimulus motion, using a scleral coil to record eye movement.

Motion of a high luminance spot (0.5 deg, 1400 fL He-Ne laser) at eccentricities 3-30° within the hemianopic field evoked pursuit in the appropriate direction. Latencies were approximately twice as long, and pursuit accelerations one-fifth to one-half as robust, as those evoked by equivalent motion in the sighted field. Step changes of spot position in the hemianopic field did not evoke accurate correction. This is in contrast with the apparent increase in threshold seen in the same group of patients (Friedman et al., this volume).

Cue counting may not be a good reflection of pursuit gain in these patients; the results also suggest differences in error processing between schizophrenics and normals. Supported in part by USPHS Grant # MH1664.
NEW ASPECTS OF DEFECTIVE PURSUIT IN SCHIZOPHRENICS

There have been few studies of the responses of pursuit eye movements of schizophrenics. We examined the tracking of S/V targets by neuroleptic-treated schizophrenics (30% of normal), measuring time-weighted pursuit gain, catch-up saccades (CUS), mean duration of tracking segments, linearity of those segments, and right-left gain ratio.

Mean gain for the schizophrenics was lower (.88 vs .99, p<.001, Mann-Whitney U) in agreement with previous reports. Mean reaction time was also longer (262.6 vs 96.6, p<.002) and mean segment duration lower (450 vs 710 ms, p<.001) for 3 cycles of tracking in the schizophrenics.

The most novel finding was the significantly poorer linearity of the pursuit segments made by the schizophrenics (.947 vs .989, p<.002). Thus, tracking deviated around a straight line without causing sufficient position error to elicte a saccade. This suggests that control of eye velocity is poorer in schizophrenics. Thus, schizophrenic pursuit tracking is grossly defective as measured by average gain. It is also defective in fine control as measured by linearity. Supported in part by USPHS Grant # MH61648.

SENSORY AND MOTOR EFFECTS OF MONOCULAR DEPRIVATION ON THE OCULOMOTOR RESPONSES IN SQUIRREL MONKEYS: L. Behrens* and O. J. Grinnell, (SPON: European Brain and Behavior Society). Dept. of Physiology, Freie Universitaet Berlin, Adenauerallee 22, D-1000 Berlin 33, Germany

By means of the electromagnetic search-coil technique the optokinetic nystagmus was measured in three normal and four monocularly deprived squirrel monkeys (Saimiri sciureus) induced within 2 to 3 days a complete immobilization of all extracellular muscles lasting for about 10 to 12 days and recovering thereafter to normal mobility of the eye within another 10 days. This technique provides a simple method to study the open loop optokinetic nystagmus (OKN). Eye movements were recorded by means of the electromagnetic search coil technique. All experiments were performed in awake squirrel monkeys.

1) In normal squirrel monkeys gain of horizontal OKN reached values between 0.8-0.97 at stimulus angular velocities below 150 degs/s (2.37 or 15° black and white vertical stripe pattern).

2) Optokinetic stimulation of the immobilized eye led to vigorous OKN and OKAN of the other eye, whereby maximum gain was found at low retinal stimulus velocities Vr (2-5 degs/s), gain above 300.

3) Increasing Vr led to a decrease in gain with a slope of approximately -20 dB/decade.

Thus, when a stripe pattern suddenly appeared, rotating at a constant angular velocity, the open loop OKN gain increased in time and reached a maximum about 40-50 seconds after stimulus onset. Thus, open loop OKN gain is a time dependent function.

The work was supported in part by a grant from the DFG (Gr 161).


The optic tectum of the barn owl contains a map of space that registers the positions of visually-stimulating objects and encodes the head heading vector needed to center the stimuli in the field of view. The sensory maps in the tectum derive from topographic projections from visually and acoustically active areas in the brain. This is illustrated by the two maps that form the basis of the present study. One is a spatial map of the visual field derived by peristimulus time histograms (PSTHs), the other is a spatial map of the auditory field. Each map contains a topographic representation of the other, which shares common features. The two maps are linked by the inferior colliculus (IC), respectively (J. C. Neurol. 218). In young owls, loss of visual/acoustic registration produces by prisms or ear plugs is corrected by compensatory shifts in the acoustic map if an eye movement is provided (Science 230).

4) When a stripe pattern suddenly appeared, rotating at a constant angular velocity, the open loop OKN gain increased in time and reached a maximum about 40-50 seconds after stimulus onset. Thus, open loop OKN gain is a time dependent function. The work was supported in part by a grant from the DFG (Gr 161).
The primate smooth pursuit system uses visual motion information to initiate and guide tracking eye movements. Lesion and electrophysiological studies have identified several brain regions that are involved in this behavior, but it is difficult to draw conclusions about their roles because the actual signals used by the pursuit system are not known. To assess the role of particular visual motion signals, we have used behavioral data as the basis for a computer model of the pursuit system.

We measured the influence of pursuit in Rhesus monkeys using the scleral search coil technique. Since analysis of the first 200 ms of pursuit indicates that there are at least three components in the response to moving targets, the model contains three parallel visual pathways. The first pathway is sensitive to image velocity, the second to image acceleration, and the third to the impulse of image acceleration that accompanies the onset of target motion. The model was tuned by adjusting each pathway so that the rising phase of the response to constant velocity and acceleration targets matched the data obtained from monkeys. The model does not explicitly reconstruct target velocity; instead, eye acceleration is driven directly by different aspects of visual motion. The model has several interesting emergent properties. First, the classical behavior of the model closely matches closed loop data, even though the model was based strictly upon the rising phase of open loop data. Second, the model shows steady-state oscillations of the same frequency as seen during sustained tracking in the monkey. Driving either the model or the monkey's pursuit system at this resonant frequency results in a 180 degree phase shift between eye and target. Finally, increasing the delay of visual feedback in the model increases the period of oscillations just as in the experimental data. The combination of these emergent properties by experimental data indicates that the elements within the model capture important aspects of signal processing within the smooth pursuit system. (Supported by NIH grant EY08378)

320.17
SHORT TERM POTENTIATION AS A MECHANISM FOR A CENTRAL INTEGRATOR L. Shen* (SPON: R. Poppele) Lab. of Neurophysiology, Univ. of Minnesota, Minneapolis, MN 55455
Neurophysiological studies in oculomotor systems suggest that a time integral operation is performed by neural circuits. Previous models of the neural integrator incorporate positive feedback to generate the required time constants. Such models are generally unsatisfactory due to stringent parameter requirements and inherent instability. A new model using presynaptic potentiation phenomena is proposed. It is shown that under certain conditions, the non-linear relation between presynaptic input and postysynaptic output has the property of an integral operator. Unlike previous models, this model is robust and without unstable singular points. Fluctuations of the parameters involved account naturally for the observed variations in the behavior of neurons in the literature. Possible incorporation of the model in the context of the vestibulo-ocular reflex is discussed. It is shown that the model fits within the three neuron pathway. The position signals commonly observed in the vestibular neurons can now be explained by synaptic interactions without the extra pathways usually assumed. Results of lesion studies on cerebellum and brain stem are also accounted for by the model. It is emphasized that the non-linear synaptic interactions between neurons have potentially very powerful computational capacity.
Supported by grant BNS 850714 from the NSF.

SENSORY SYSTEMS: AUDITORY SYSTEMS VI

321.1
Previous investigations in our laboratory observed changes in cochlear blood flow (CBF) in non-normoxic Wistar-Kyoto (WKY) rats that could be interpreted as autoregulation of CBF. To test this hypothesis WKY rats were intra-arterially infused with high doses of phenylephrine (0.02 mg/kg/m in 0.15 M NaCl) or angiotensin II (1000 pmol/kg/min in 0.15 M NaCl) for 10 minutes. The question of whether autoregulation of CBF could be induced while systemic blood pressure was maintained at baseline levels, was addressed by infusing both vasoactive compounds simultaneously into the supplying vessels of the cochlea to induce local changes in CBF independent of changes in systemic blood pressure. This was accomplished by inserting a micropipette (external diameter approximately 175 microns and internal diameter approximately 125 microns) into the vertebral vessel to allow slow infusions (100 nI/min) of angiotensin II, phenylephrine or vasopressin. The results showed that CBF decreases and are suggestive of local vasoconstriction caused by vascular receptor binding in the supplying vessels of the cochlea.

321.2
SINGLE CHANNEL PROPERTIES OF GOLDFISH (Carassius auratus) AUDITORY NEURONS IN VITRO. R.M. Davis, E.A. Mount and W.P. Seweli. Eaton-Peabody Lab., Massachusetts Eye & Ear Infirmary, Boston, MA 02114.
The goal of this work is to characterize the membrane properties of auditory neurons in culture. Single auditory nerve fibers were microdissected from the sacular nerve of the goldfish without the use of proteolytic enzymes and maintained in culture for periods of up to four weeks at room temperature. The patch clamp procedure was used to record single channel currents from the growing ends of the neurons as well as from axonal membrane that had been acutely removed from the surrounding myelin. Experiments using the whole cell and the single channel techniques have revealed ionic channels with conductances ranging from 16 to 120 pS in solutions that select for K+ (CaCl2, KCl). These data are being systematically categorized according to voltage dependence, ionic specificity and position along the length of the neuron.
Scanning electron microscopy was used to define regional differences in sacculare morphology and efferent innervation. Previous investigations in our laboratory demonstrated that spontaneous activity of lateral line nerve fibers of X. laevis is inversely related to the concentration of elevated K across the membrane at -30 mV. The translocation rate of 141 ± 9.7 (n=10), 143.0 ± 10.3 (n=7) in MC; 78.1 ± 4.1 (n=30), 143.7 ± 10.3 in SM. On the basis of the electrochemical potential gradient calculated from the present results, the transport mechanism of monovalent ions in the toadfish was investigated.

Supported by NIH #5R01-NS14538 from the NICHD.

Supported by the Medical Research Council, U.K.

**321.6**

**HAIR CELL AND PRIMARY AFFERENT RESPONSES IN AN IN VITRO PREPARATION OF THE ALLIGATOR LIZARD COCHLEA. R. A. Eatock. Dept. of Physiology, University of Rochester, Rochester NY 14642.**

Understanding of stimulus processing by hair cells has benefited greatly from in vitro preparations that provide access to the hair cells' mechanosensitive surfaces. We have extended this kind of preparation to include the primary afferent (cochlear) neurons, using the auditory organ of the alligator lizard as a model system. The organ is excised and maintained at 20-23°C in an artificial perilymph (in mM: 168 or 164 Na+, 2 K+, 2.5 Ca2+, 10 Mg2+ and 10 HCO3-; pH=7.4). Intracellular potentials are recorded from hair cells and afferent processes of the primary afferent neurones.

As in other preparations, the hair cell responses may be classified into two main types based on the threshold for stimulation and their sensitivity to various drugs. In this study, we have focused on the primary afferent response and its dependence on the concentration of extracellular K.

Intracellular potentials were recorded from hair cells of the bullfrog sacculus. The sensory epithelium is stripped of its overlying otolithic membrane and the apical surface gently pressed onto nitrocellulose paper (NCP) with a force of 5-15 millinewtons. The preparation was then incubated in an artificial perilymph (in mM: 168 or 164 Na+, 2 K+, 2.5 Ca2+, 10 Mg2+ and 10 HCO3-; pH=7.4) for 30 min. The primary afferent response was then recorded using an autodrive manipulator at the rate of 1-2 µm/sec. The changes of the membrane potential and the electrochemical potential gradient calculated from the present results, the transport mechanism of monovalent ions in the toadfish was investigated.

**321.7**

**REGIONAL DIFFERENCES IN SACCULAR MORPHOLOGY AND EFFECTIVE RESPONSES IN THE TOADFISH, Opsanus tau. A. Steinacker and D. G. Drescher. Lab. of Bio-otology, Wayne State Univ., Detroit, MI 48201.**

Our results suggested that transmitter can be released from hair cells in the lateral line without the influx of Ca through voltage-dependent Ca channels (VSCC). The effect of D-600 on spontaneous activity was studied on isolated hair cells of X. laevis. The effect of D-600 on spontaneous activity in 1 mM K was 32% of spontaneous activity in 3 mM K, and D-600 inhibited spontaneous activity completely. We propose that preceding K increases firing rate by depolarization of the hair cells, which increases Ca current through Ca channels and D-600 sensitive release of transmitter, while in reduced K (hypopolarizing) and/or reduced Ca, spontaneous activity is sustained by a mechanism which is less sensitive to D-600. Higher Ca concentration can reduce spontaneous activity further; indeed D-600 is known to inhibit many processes unrelated to VSCC at concentrations greater than 1 uM. Miller & Freedman, Life Sci. 34:1205-1211, 1984. (Supported by NIH NS16166 to DGD.)
321.11 TUNING PROPERTIES OF TUBEROUS ELECTRORECEPTORS. E.S. Olsson, I. Norden, and E.E. Holosp (SPON L. Physiol. Dept. of Physics and Elec. Eng., M.I.T. Cambridge, MA 02139). We measured the frequency dependent impedance of small areas of the electroreceptor tuft structure of a Pacific stargazer fish, and used the data to make an equivalent circuit model of the structure. This model assumes that the hair bundle is a mass-spring system and that the electroreceptor cell body is a fixed point. The data obtained were fitted to a loss-free model, and the fitted parameters were used to calculate the response of the model to a range of frequencies. Our measurements usually supported the passive linear system model. However, stimulated electroreceptor voltage oscillations were detected in some measurements, indicating that the electroreceptors sometimes operate in a regime of active nonlinearity. Nonlinear analysis of the Hodgkin-Huxley type of model that we used for the membrane impedance showed that it was possible to fit the data. When driven with a current frequency, the spontaneous oscillations could be reduced by increasing the amplitude. These results suggest that weak spontaneous oscillations do not degrade sensory reception.

321.14 EFFECTS OF HEMICHOLINUM-3 ON BRAINSTEM AUDITORY EVOKED POTENTIALS IN THE RAT. C. Knapp, C. Wirtz-Brugger, R. Cornfeldt, and S. Fielding, Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876. Acetylcholine (Ach) has been implicated in the brainstem auditory evoked potentials (BAEP) in the rat. Hemicholinium-3 (HC-3) inhibits Ach synthesis by blocking the high affinity uptake of choline and thereby causes depletion of Ach required for cholinergic transmission. The purpose of the study was to provide proof for direct Ach involvement in the generation of the BAEP waves. HC-3 was administered once to the rat. The following compounds were evaluated in a repeated measurement design: Ach (10 μg/mg) decreases BAEP waveforms; Physostigmine (P) 0.01 mg/kg ip and carbamylcholine (C) 0 μg/mg both increased the BAEP VI significantly. C pretreated with HC-3 reversed the effects of HC-3 and significantly increased BAEP waveforms. P did not alter decreases caused by HC-3 suggesting a direct involvement of Ach in the BAEP.
321.15


Three experiments evaluating the effects of various stimulus manipulations on the click-evoked gerbil BAER are reported. Five replicable peaks (i through v) are observed within six ms of click onset. Click polarity and level do not affect the amplitudes. Amplitude is smaller after a 0.09 ms click than after a 1.5 ms click. A parallel increase in peak latencies and a decrease in peak amplitudes with decreasing click level, Exper. 1. 2 covared click level with age. Peak latencies increased with increasing click repetition rate; this latency increase was greater for the later BAER peaks, producing an increase in the l-v interval. As rate increased, the amplitudes of waves i and v decreased monotonically, whereas the amplitudes of waves ii, iii, and iv were largely unaffected by click rate. For both experiments, the amplitudes of waves i and v decreased monotonically, whereas the amplitudes of waves ii, iii, and iv were largely unaffected by click rate.

321.17


Anatomical changes in the auditory periphery, central pathways, and cortex occur with age. Non-invasive electrophysiological measures of age-related changes, especially changes in auditory cortex, would enhance the understanding of the normal aging process and increase ability to monitor aging and recognize abnormalities.

We studied click-evoked AEP scalp topographies (20-256 μV) in 20 normal human male and female subjects between the ages of 18 and 80 years. The amplitudes of waves i and v of the CAP were measured using single unit techniques and suggest that the ANN can be used to accurately determine the τA and τR of auditory fibers using low frequency, low amplitude, long duration tones. It has become possible to record ANN responses to 300ms probe tones at Δt=0 the recovery of the response as a function of Δt (0). Δt is the delay between masker offset and probe onset, and τR is the recovery time constant. For 300ms probe tones at Δt=0, the recovery of the response as a function of Δt (0) is described by the equation R(Δt)=(100-ω)e-Δt/τR (Harris & Dallos, 1979).

321.18


The neural origin of the brainstem auditory potentials and their potential interactions with other nervous elements are still uncertain. In particular, the role of various neural elements (cells, fibers) in the auditory brainstem response (ABR) has not been decided. In order to provide information to resolve these problems, we made lesions in the brainstem auditory pathway of the cat that were destructive of well defined brainstem auditory fibers (kainic acid) or the myelin of nerve fibers (Lyso phosphatidyl choline-LPC).

321.19

ADAPTATION AND RECOVERY OF AUDITORY NERVE NEUROPHONIC (ANN) RESPONSES TO T. G. CHIMENDE and CLEON COLEMAN, Epstein Laboratory, University of California, San Francisco, CA 94143.

Determining the time constants of adaptation and recovery for long duration, low frequency tones in the auditory nerve is an essential part in the understanding of processing of sustained acoustic signals. ANN responses to 100Hz, 100ms long, 10dB SPL tones under anesthesia, had before tone onset the 1st peak waveform that was followed by the 2nd peak waveform at a latency of 0.7 ms and an amplitude of 1.25 μV. The latency of the 1st waveform decreased about 0.5 ms in the 1st ms after the tone onset.

This study addresses the question of whether a new non-NMDA antagonist, 3-dihydroxy-7-nitro-quinoxaline (CNQX/FG9065; Ferrosan), affects the CAP. Perilymph spaces of guinea pig cochleas were perfused with Ringer solutions containing up to 500 μM concentrations of CNQX at a rate of 2.5 μl/min. For 10 min. Immediately after each period of perfusion cochlear potentials were recorded from a wire inserted in the basal turn scala vestibuli. CNQX as low as 3.3 μg suppressed the CAP. CM and SP were not affected.

321.20

THE MULTIPLE GENERATORS OF THE AUDITORY MIDDLE LATENCY RESPONSE. H. Kraus, T. McGee, Michael Reese Med Ctr and Univ of Chicago, Chicago, IL 60616.

In the guinea pig and gerbil, auditory evoked middle latency (MLR) components recorded from the midline differ from those recorded over the temporal lobes. These differences are apparent with: intracortical injection of neuroinactivating agents (Tidocaine and kainic acid), temporal lobe ablation, electrolytic lesions, systemic anesthesia, stimulation rate, and maturation.

Data reveal that midline and temporal lobe MLR components vary independently, suggesting different source of excitation from different generator sources. The particular orientation of the generators responsible for the MLR in the guinea pig and gerbil facilitates the identification of individual components, whereas in humans, MLR component Pa is distributed over widespread areas of the cortical surface, possibly making the activity of the multiple generators likely to underlie the response. Our data support the existence of multiple MLR generators in laboratory animals and provide insight into the generators of the MLRs in humans.

The different course of development observed in midline versus temporal lobe components may help explain why the MLR is inconsistently obtained in children. The latency of the response in humans may occur because one of the generators, presumably the temporal lobe generator, has not yet developed, although other generators may have already matured. (Supported by NIH-NINCDS RO1 NS 21150.)
322.4 COMBINED SUBTRACTION/DIFFERENTIAL HYBRIDIZATION SCREEN FOR GENES INDUCED IN OPTIC NERVE INJURY. M.E. LaBate* and J.H. P. Skene, Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305-4408.

Direct analysis of axonal proteins by several laboratories has identified a small number of proteins whose synthesis is widely correlated with axon growth. In the present study, we have used combined subtractive/differential hybridization as a general screen for genes induced during optic nerve regeneration in a large-eyed goldfish. The brain was prepared from control retinae (C) and from 'regenerating' retinae taken 10 days after optic nerve crush (R). Each of these cDNAs was then hybridized to an excess of control poly A+ RNA to a R/C of 800. The cDNA remaining unhybridized in each reaction (C - C and R - C, respectively) was then used for two more hybridizations to two different cDNA libraries prepared from regenerating retinae. Of 100,000 clones, 90 (0.09%) gave a moderate to strong differential signal with the subtracted 'R - C' probe compared to that seen with the subtracted 'C - C' probe. Work is now in progress to determine the number of different mRNA sequences represented in this group of clones.

The identity of one mRNA induced during fish optic nerve regeneration was established using a rat GAP-43 cDNA probe. A 1.4 kb rat cDNA selected with the rat GAP-43 probe hybridiizes to Northern blots to a 1.4 kb mRNA that is strongly induced in fish retina after optic nerve injury. Preliminary sequence analysis indicates that the amino-terminal portion of GAP-43 mRNA encodes a putative membrane-binding domain, are highly conserved between rat and fish.

Supported by NIH grants NS-09015 and NS-14957 and a fellowship from SCRF/PVA.
322.7 REGENERATION: GAP-43
REGULATION OF GAP-43 GENE EXPRESSION DURING AXONAL REGENERATION IN SENSORY NEURONS. G. Basset* and L.H. Paton Skene (Spon: T. W. Kraft). Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

GAP-43, a neurotrophic homologue of growth cone membranes and certain mammalian pre-synaptic terminals, is expressed in correlation with both regenerative, and developmental axonal outgrowth in a broad evolutionary range of organisms. In the rat, GAP-43 is encoded by a single-copy gene whose expression is regulated primarily at the level of transcription. In order to further analyze GAP-43 gene expression during regeneration, we have examined its induction in the sensory neurons of the L4 and L5 dorsal root ganglia (DRG) in response to peripheral nerve injury.

Using a rat GAP-43 cDNA clone (Bass et al., 1991, Cell 49, 785-791) to probe Northern blots of RNA isolated from crush and control DRG, we have observed that GAP-43 expression is induced in the cell bodies after an injury. GAP-43 gene expression rapidly rises to a maximum by 3-4 d post-injury, and is maintained at this level for at least 28 d post-injury. Neither the magnitude, nor the timing of this induction is affected by the nature of the injury (crush versus cut), nor by removal of a 12 mm segment of the distal stump. The expression declines to control levels between 28-40 d post-injury. However, if successful regeneration is prevented by a cut injury, or a cut in conjunction with removal of the distal stump, GAP-43 mRNA does remain elevated for a longer period of time (up to 50 d following nerve transaction). Furthermore, the data analyzed thus far indicates that this timing of GAP-43 gene induction is not dependent on the presence of the cell body, but is dependent on the presence of the axon.

The early induction of GAP-43 gene activity is consistent with the hypothesis that it is a pre-requisite for, rather than a consequence of, axonal outgrowth. In order to more definitively address this issue we employed colchicine injection into the injury site to disrupt axonal outgrowth. Using doses of colchicine which were effective in blocking retrograde transport of a fluorescent tracer, we have observed that injection of colchicine (via the nerve does not a) delay the induction of GAP-43 gene activity by an injury; b) induce GAP-43 gene activity by itself in the absence of a crush. Thus, our observations indicate that GAP-43 expression precedes axonal outgrowth.

This work was supported by NIH grants NS 08096 (GSB) and EY 07379 (HPS).

322.8 GAP-43 INDUCTION IN REGENERATING DORSAL ROOT GANGLION CELLS: AN ANALYSIS OF SORTING IN AXONAL TRANSPORT. D.L. Schum and J.H.P. Skene. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

In addition to eliciting the regenerative response of the peripheral branch of dorsal root ganglion (DRG) axons, sciatic nerve injury also enhances the propensity of the central branch of DRG axons to regenerate through sciatic nerve grafts within the spinal cord (Richardson, P. M. & Verge, V. M. K., J. Neurosci. 19, 858-869, 1990). It is of interest to determine if a cellular response to injury including the production of axonally transported growth-associated proteins (GAPs) affects the growth propensity of a distant, uninjured axon branch. Thus, we have used Western blot analysis of central and peripheral branches of DRG axons to determine if increased levels of the protein GAP-43 are involved in the enhanced regenerative propensity of central axon branches in response to peripheral injury, or whether newly synthesized GAP-43 is routed exclusively to the injured peripheral axon branch.

Adult rats underwent a unilateral sciatic nerve crush below the sciatic notch and survived 2-8 days. Membrane fractions were prepared from homogenized tissue taken from three areas: the sciatic nerve distal to the L4 and L5 DRG, the L4 and L5 DRG themselves, and the dorsal roots at L4 and L5 proximal to the ganglia. Membrane fractions were solubilized, electrophoresed, electroblotted to nitrocellulose, and probed with a monoclonal antibody specific for GAP-43.

Within 2 days following sciatic nerve crush, GAP-43 immunostaining on Western blots is elevated in comparison to uninjured control tissue in peripheral sciatic nerve, in DRG, and in central dorsal root segments. The amount of GAP-43 detected in all three areas increased in subsequent days. The increased amount of GAP-43 synthesized in DRG cells following sciatic nerve crush is thus not exclusively transported to the axon branch sustaining the injury. Transport of induced GAPs to the central branch of DRG axons may therefore be crucial to the enhanced growth ability of central DRG axons that follows peripheral lesions.

322.9 ELEVATED GAP-43 IN SPONTANEOUSLY REGENERATING CNS AXONS OF ADULT RATS. A. P. Forster. Department of Neurosciences, McMaster University, Hamilton, Ontario L8W 3J5.

Evidence for a spontaneous regeneration of severed axons which formed detours around lesions (made and marked by the implantation of a fine wire cutting device in the brain), that was entirely histological (J. Comp. Neurol. 210:135, 1982), has now been extended by a correlative study with monoclonal antibodies to a neuron-specific growth-associated protein, GAP-43 (provided by J.H.P. Skene and D.J. Schreyer) and to neurofilaments (210 kDa. Pentobarbital anesthesia was used). Immediately post lesion, neither an axonal detour nor elevated GAP-43 was observed. Axonal detours then increased progressively as the number of severed axons facing the lesions was reduced. Within a few days. GAP-43 was elevated in the terminal portions of severed axons, and was observable for at least 3 weeks in those pursuing a reconstructed course, but not in the lesions. Within the population of axons now curving around the lesion, GAP-43 was elevated primarily in those close to its end. I.e., the most recent arrivals, suggesting that it increases early in the regeneration and falls thereafter. There was no evidence (swelling, varicosities) in the detouring axons of mechanical hindrance of axoplasmic transport.

These immunocytochemical findings support the anatomical evidence for the occurrence of spontaneous axonal regeneration after lesions of in the adult rat brain.


After nerve transection, Schwann cells (Sc) in the distal nerve stump undergo a series of poorly characterized events which may influence neuronal regeneration. Using immunocytochemical methods, we examined the expression of known, Sc-synthesized antigens which are localized in the cytoplasm or on the membrane. The immunoreactivity of glial filaments (GAF), a cytoskeletal component specific in ensheathing Scs, diminished after injury. In contrast, the immunoreactivity of synaptophysin (SYP), a cytoskeletal component in myelinating Scs, became prominent within proximal Scs. Upon reinnervation, GAF-positive filaments increased in number and length within finite domains of the nerve. Synaptophysin (SYP) became undetectable in some of these areas, whereas it remained constant in others. In control or unjured nerves, SYP and GAF were undetectable. The latter was associated with an unidentified component of the Sc cytoskeleton. S-Y100 protein, normally not present in Scs, became undetectable in the distal nerve stump and gradually increased during reinnervation. These studies indicate that alterations in Sc-neuron contact result in modifications in the expression of Sc proteins which can in turn influence Sc shape and function. Supported by PHS 8011 1989.

323.2 IDENTIFICATION AND CHARACTERIZATION OF A FACTOR WHICH INHIBITS POSTTRANSLATIONAL MODIFICATION IN LIGAND-INDUCED NERVE REGENERATION. W.J.* G. Chalhoubny*, S. Byrne-Atthal and N.A. Troppila, Dep't of Physiology, New Jersey Medical School, Newark, N. J. 07103.

The posttranslational modification of proteins by amino acid addition has been demonstrated in a variety of biological systems. In our laboratory, we have found that these reactions take place in rat sciatic nerves, are greatly amplified two hours after crush injury and that the addition of individual amino acids appears to be regulated by specific factors (Shyene-Atthal et al., 1986, Science, 231, 603-605). In the present study we have used Western blot analysis of central and peripheral DRG cell fractions two hours following nerve transection to characterize the inhibitor to posttranslational lysine addition at two hours after crush injury to rat sciatic nerves.

In order to characterize the inhibitor, the inhibitor was shown to be heat stable (95°C, 5 min.), and sensitive to treatment with trypsin (0.1% trypsin caused a 50% reduction in inhibitory activity). These findings suggest that the inhibitor is a histidine protein. When the inhibitor was passed through a Sephadex G-50 column, inhibitory activity eluted with molecular weight standards of approximately 10-20 kDaltons. However, following further purification on a MONO R5 12.5 SEC exclusion column, the inhibitory factor appeared to have a molecular weight of less than 4,000.

In summary, the inhibitory factor of posttranslational modification of protein in nerve regeneration is a small molecular weight factor which is heat stable and trypsin sensitive. Further characterization of this molecule is in progress. (Supported by grants from the NID).
REGENERATION: OTHER GROWTH-ASSOCIATED PROTEINS

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Medical School and VA Medical Center, West Roxbury, MA 02132.

We previously reported on the late occurrence of NF phosphorylation in development and regeneration. Here we show that NF phosphorylation is a complex phenomenon in developing and regenerating NF phosphorylation events may occur shortly after NF expression or follow a considerable delay. NF phosphorylation was studied by indirect immunofluorescence with antibodies to phosphorylated epitopes. The antibodies either detected NFs shortly after their appearance (as indicated by double labeling experiments with NF polyclonal antibodies) or after a considerable delay, ranging from 4 to 9 days in embroyonal development, from 6 to 15 days in sciatic nerve regeneration and from 12 to 27 days in primary cultures. With most (but not all) antibodies, there was a good correlation between in vivo and in vitro findings as to the early or late appearance of phosphorylated epitopes. One monoclonal antibody stained regenerated axons one month after transaction with an abnormal pattern, thus suggesting differences between normal and regenerating nerves with respect to NF phosphorylation. Supported by the Veterans Administration.


We have seen that the majority, but not all, of those proteins rapidly transported along frog optic nerve fibres are also conveyed at the fast rate along rat optic nerve fibres, and that a similar pattern also exists in rat optic nerve axons. Following crush of the frog optic nerve the labelling of many of the fast transported proteins (FTP) increases. Most notable among these changes was the large increase in labelling of a GAP-43 like protein in the regenerating frog optic nerve. Also, the labelling of another FTP, designated A25, which was also present in the normal optic nerve, and in primary dissociated cultures of spinal cord neurons, increased considerably in the regenerating frog optic nerve. Shortly after crush of the rat optic nerve there is a large increase in the labelling of a transported protein of similar molecular weight and isolectric point to GAP-43. A very interesting similarity between crushed frog optic and sciatic nerves was the appearance of a polypeptide, designated A25, seen previously to be generated specifically at the site of damage in the sciatic nerve. The presence of A25 is most likely a fast axonally transported protein, possibly a high molecular weight precursor, designated A25, which was also present in the normal optic nerve. However, A25 but not A25 was also seen in the patterns of FTPs delivered to the terminals of the optic nerve axons, that is in the frog optic foci. This suggests that A25 is not present in normal optic nerve or its terminals, and its appearance in the nerve is damage specific. A polypeptide with similar molecular weight and isolectric point to A25 was also seen in the rats optic nerve shortly after crush. The high molecular weight protein, A25, also appears to be rapidly transported in normal undamaged rat optic nerves.

This work was supported by NIH grant EY014449; GSP is a Markey Fellow.


The structural hypothesis of slow axonal transport (SAT) suggests that regenerating axons derive their cytoskeletal proteins from those previously in the axon, and moving along it at 4 mm/day. However, the synthesis of cytoskeletal proteins by newly-transported NFs are synthesized in the cell body increases within a few hours after axonal injury, which seems unnecessary if regeneration is sustained by pre-formed proteins.

The I5 DRG of anesthetized adult rats was labeled with 35S-methionine 3 days after crushing the sciatic nerve and 60 minutes post injury. The DRG. Labelled proteins present in thenerve distal to the crush were analyzed two days later: these included actin and tubulin immunoreactive epitopes. In the cell body, cytoskeletal proteins synthesized in the cell body 3 days after injury are transported into the regenerating axons, over 60 mm distal from the cell body, at a velocity of at least 30 mm/day.

Our results demonstrate that regenerating axons do incorporate cytoskeletal proteins produced by the cell body post-injury and raise the possibility that these may be special variants of the normal cytoskeletal proteins, which are required for axonal outgrowth.

(Supported by MRC and AMFMR).

323.6 LEVELS OF B-PREPROTACHYKININ ($\beta$-PPT) mRNA AND TACHYKININS CHANGE DIFFERENTIALLY IN RAT DORSAL ROOT GANGLIA (DRG) FOLLOWING SCIATIC NERVE SECTION. D.B. Henken, A. Tesileac, M.F. Chestelet and M. Murray. The Medical College of Pennsylvania, and the VA Medical Center, Philadelphia, PA.

Localization of a peptide and the mRNA for that peptide within specific populations of DRG neurons was used to examine metabolic changes associated with axotomy and regeneration. In DRG, both 20% of the total neuronal population contains tachykinins and the mRNA for the tachykinin precursor, $\beta$-PPT. It is known that sciatic nerve section at first reduces levels of tachykinins in DRG which later recover when regeneration is complete. We examined levels of tachykinins and $\beta$-PPT mRNA 2 weeks and 6 months following sciatic nerve section and re-appearance of both the mRNA and peptide returned to control levels of 15-20%. These results suggest that regulation of metabolic changes during regeneration depends at least in part on alterations in gene expression and in that in situ hybridization histochemistry can be used to study this regulation. Supported by NSF grant BNS860641, VA Medical Research Service, NIH grant NS24707 and USAMRICD grant 5193002.


We have previously shown that regenerating axons from the tiger salamander retina can be maintained in vitro where they regenerate processes and form synaptic connections. In synaptic vesicles during neurite regeneration, a monoclonal antibody to the synaptic vesicle protein SV2 (Buckley and Kelly, J. Cell Biol., 100:1284 1988) was used in combination with indirect immunofluorescence. Cells were isolated by enzymatic digestion and mechanical dissociation. Rod, cone, and bipolar cells examined immediately after plating had light perinuclear fluorescence and intense staining synaptic terminals, consistent with vesicle accumulations observed by electron microscopy. Multinuclear neurons, which include horizontal, amacrine, and ganglion cells, retained only the proximal portion of their processes and showed only dim perinuclear staining. Intense staining remained in photoreceptor and bipolar terminals for at least 2 weeks; retention of SV2 staining did not depend on the development of cell contacts. In addition, process outgrowth from bipolar neurites was biphasic immunoreactive. In contrast, new processes from ganglion cells, identified by retrograde labeling, stained only slightly. However, a small percentage of unidentified multipolar cells showed punctate staining in processes. Thus, retinal neurons isolated with axons retain SV2 staining in synaptic terminals and appear to regenerate SV2-rich processes. These findings suggest that synaptic vesicle protein SV2 in rat and tachykinin receptors can be maintained in the absence of postsynaptic cell contact.


323.8 CALCIUM ACTIVATED NEUTRAL PROTEASE (uCANP) ACTIVITY IN AXONS IS INCREASED PROXIMAL TO A NERVE CRUSH. D.J. Fink and M. Mata, Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

CNPAs are cysteine endopeptidases present in the cytosol which require calcium ions for activity. A CANP activated by millimolar $\text{Ca}^{2+}$ has been identified in peripheral nerve and degrades neurofilaments in Wallerian degeneration. uCANP, activated by micromolar $\text{Ca}^{2+}$, has also been identified in peripheral nerve, although its role is not known. We measured uCANP activity in normal nerve and in proximal axons 1 wk after distal nerve crush.

Neurofilaments (NFs) were isolated using a modification of the method of Schlaepfer and the CANP activity in the supernatant determined by the decrease of 14C casein in the presence of 50 mM $\text{Ca}^{2+}$. The enzyme has an apparent Vmax of 70 ug casein/mg crude CANP/30 min and a Kms of 25uM. The activity is linear for 30 minutes, and using our standard incubation conditions varies directly with the amount of CANP.

We found activity in normal nerve was 41 ug casein/mg CANP/30 min. The activity of crude CANP increased from proximal spinal cord nerve 1 wk after distal nerve crush was 71.1 mg casein/cANP/30 min (P < 0.05). Incubation of tritium labeled NF with 10ug of CANP for periods ranging from 1-24 hr showed that uCANP activity in the nerve 1 wk after crush degraded the NF more rapidly than the normal uCANP.

The presence of uCANP in normal nerve suggests that turnover of NF may occur within the axon during normal axonal growth, and agrees with the presence of NF immunoreactive breakdown products in normal axons. The increased activity of uCANP in proximal nerves after crush may be related to the changes in NF transport which occur after axotomy.

Supported by VA Merit Review Grants to Dr. Mata and Dr. Fink.

In contrast to higher vertebrates, the CNS of lower vertebrates has the capacity for functional nerve regeneration after injury. A useful model to study this phenomenon is the goldfish visual pathway which regenerates following optic nerve crush. After unilateral optic nerve crush, PA activity appeared in crude homogenates of the crushed nerve. No activity was observed in the contralateral uninjured nerve, nor in sham operated controls. The PA activity was seen as early as one day post-crush, reaching a peak at about 10 days; by 80 days post-crush, at which time vision is restored, the activity is no longer detected. Electrophoretic zymography for PA activity revealed the presence of a major species at 75K with two other species of variable intensities at 65 and 38K. The PA activity could be partially inhibited with polyclonal antibodies against either human tissue-type PA or human urokinase-type PA.

These results demonstrate that PA is present in the goldfish and its expression is correlated with the process of optic nerve regeneration. (Supported by grants EY05212 (NIH) to NS and HD17875 (NIH), BC525H (ACR) to BS.)

323.9 REGENERATION: OTHER GROWTH-ASSOCIATED PROTEINS

Nerve growth factor is a polypeptide neurotrophic agent that is produced during sciatic nerve regeneration. M. De, B. De, M. D. B. De, and G. W. Kreutzberg (Spon: J. Noth). Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

We have studied the role of optic nerve regeneration of the protease plasminogen activator (PA), which has been previously implicated in nerve regeneration. After unilateral optic nerve crush, PA activity appeared in crude homogenates of the crushed nerve. No activity was observed in the contralateral uninjured nerve, nor in sham operated controls. The PA activity was seen as early as one day post-crush, reaching a peak at about 10 days; by 80 days post-crush, at which time vision is restored, the activity is no longer detected. Electrophoretic zymography for PA activity revealed the presence of a major species at 75K with two other species of variable intensities at 65 and 38K. The PA activity could be partially inhibited with polyclonal antibodies against either human tissue-type PA or human urokinase-type PA.

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Retinal proteins that are synthesized in greater amounts following axotomy of the goldfish optic nerve can serve as biochemical probes for understanding the molecular mechanisms underlying CNS regeneration. Such proteins can be radiolabeled and then identified in gels of regenerating optic nerve, indicating that they originate in retinal ganglion cells (RGC's) and that they are axonally transported. We are synthesizing acidic 68/70 KDa axonally transported doublet in which the incorporation of [3H]-methionine is increased 4-5 days after nerve crush. Here we report immunocytological evidence that this cytosolic doublet is indeed localized within RGC's. The 68/70 KDa doublet was purified by DEAE and lectin chromatography of a high speed supernatant of goldfish brain. Following SD-PAGE, the proteins were electroblotted onto nitrocellulose, and strips containing the doublet were implanted subcutaneously into rabbits. Reactive antisera, judged by Western blots, was incubated overnight (diluted 1:1000) with aldehyde-fixed crystalline sections of control and 10-15 day postcrush retinas. Bound antibody was visualized by immunofluorescence (goat antirabbit coupled to FITC) or immunocytochemistry (avidin-biotin-peroxidase conjugate). While immunoreactivity was localized to the RGC's of both control and postcrush retinas, it was markedly increased as a result of axotomy. Preimmune serum was nonreactive. These results indicate that the 68/70 KDa doublet is synthesized and is present in increased amounts in the regenerating RGC's, and thus serves as a biochemical correlate of functional recovery in the teleost CNS. (Supported by NEI grants EY0547 and EY02148.)


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Levels of mRNAs for tubulin (Tm), actin (Am), and GAP43 increase in rat facial motoneurons after facial nerve injury, while nNOS may also be induced. The time-course of these changes in more detail to determine if failure of regeneration is associated with inability to sustain them. (Supported by MRC of Canada and AHPBR.)

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323.15
A Knoxional injury produces characteristic changes in cellular morphology, protein expression, and RNA metabolism. Thus, the regulation of the levels of expression of mRNAs for major cytoskeletal proteins in regenerative processes can be explored by examining levels of specific mRNA species at various stages following nerve injury/regeneration. To this end, in situ hybridization was used to measure levels of cytoskeletal protein mRNAs in motor neurons of the L4-L5 spinal cord at several intervals following axotomy and sciatic nerve. Fourteen days postaxotomy, levels of the low molecular-weight neurofilament subunit mRNA were maximally reduced (3.5 fold). Similar changes were seen in levels of mRNA coding for other neurofilament subunits. In contrast, levels of \( \beta \)-tubulin mRNA in neurons were increased maximally (twofold) 14 days postaxotomy. Patterns of changes in levels of cytoskeletal protein mRNAs that result from axotomy in these spinal motor neurons of the central nervous system parallel those recognized following anoxia of sensory neurons in the peripheral nervous system. These studies in animal models of regeneration will lay the foundation for studies of cytoskeletal protein gene expression in animal models of degeneration and human neurodegenerative diseases.

323.16
We have utilized peripheral and central axotomy to examine ultrastructural changes following injury in the DRG neurons of rats. The DRG neurons of rats are known to be very sensitive to injury, and to demonstrate a variety of axonal regenerative responses. We have injured the DRG neurons by stimulating them with 100 Hz for 10 sec. The DRG neurons were harvested and prepared for electron microscopy at 1, 3, 6, 12, and 24 hrs after injury. Our results demonstrate that the DRG neurons are highly sensitive to injury, and that they demonstrate a variety of axonal regenerative responses. These responses include axonal sprouting, axonal retraction, and axonal regeneration. We have also observed changes in the structure of the DRG neurons, including changes in the organization of the neurofilaments and myelin sheaths. These changes suggest that the DRG neurons are highly sensitive to injury, and that they demonstrate a variety of axonal regenerative responses.
MONOAMINES AND BEHAVIOR IV


Studies have demonstrated that the GABA agonist MUSC enhances amphetamine (AMP) and apomorphine (APO) stereotypy (STERO), and that MUSC also enhances neuroleptic-induced catalepsy (CA). We investigate possible MUSC interactions with the D1 and D2 DA receptor subtypes, we examined MUSC's effects on the STERO and CA produced by injections of D1 or D2 selective agonists.

Adult male rats were injected with MUSC (0.1-1.5 mg/kg, sc) either vehicle (VEH), the D1 agonist SKF38395 (3 or 20 mg/kg, sc) or the D2 agonist haloperidol (2 mg/kg, sc) or metoclopramide (25 mg/kg, sc). Beginning 15 min later, catalepsy (CA) was measured periodically for 90-120 min. Our results suggest that muscimol potentiates both D1 and D2 DA receptor mediated catalepsy and that the catalepsy produced by inhibition of DA agonists probably involves different neural substrates.

324.7 EFFECTS OF D1 AND D2 RECEPTOR ANTAGONISTS AND 5HT RECEPTOR ANTAGONISTS ON MEASUREMENTS OF THE BEHAVIORAL RESPONSES OF MUSCIMOL (MUSC) TO DOPAMINE (DA) RECEPTOR STIMULATION IN MICE. M. Swain*, D. Slovis, L. Thompson*, J. Pehel, and R. Eaton*. Dep. Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

The dopamine (DA) and serotonin (5HT) systems play important roles in modulating prolactin (PR) release. The contribution of D1 and D2 DA receptor subtypes to PR regulation were studied in monkeys as a model for human neuroendocrine function.

METHODS: 12 male rhesus monkeys were studied. D1 and D2 agonists included: haloperidol (H) and SCH33929 (SC), 5HT agonists included: buspirone (B), 8-OHDPAT (8OHP), ipsapirone (I), lipoxy (L), MP475 (M), and tioptophans (T) and antagonists: ritanserin (R) and metergoline (ME).

RESULTS: The relative potency (PO) (in uM/kg) for a 40 mg/ml prolactin increase and the percent increase was:

- H 200 80 40 20 10
- 8OHP 20 40 80 160 320
- 8OHP+H 20 40 80 160 320
- 8OHP+R 20 40 80 160 320
- 8OHP+ME 320 640 1280 2560 5120
- 8OHP+T 320 640 1280 2560 5120

ME + PC as well as PC alone did not synergize, thus the D1 and D2 systems appear to function independently on PR release. D1 agonists are 50 times more potent than 5HT stimulation in increasing PR. Behavioral effects including sedation (and catalepsy with H, SC and B) were marked and clear differences were observed between the drugs studied. (See J. H. Krystal, et al. this volume).


We previously shown that the classic dopamine (DA) agonist apomorphine (APO), injected into the medial preoptic area (MPOA) of male rats, facilitated several copulatory measures including increased copulation rate. The DA antagonist cis-flupenthixol (Flup) blocked APO's facilitation of copulation and, in higher doses, impaired copulation. To determine whether MPOA DA facilitation of copulation is mediated by D1 or D2 receptors, we injected either the D1 agonist LY163502, the D2 agonist SKF83256, or a combination of the two into the MPOA immediately before the animal tested.

In Exp. 1, 1 ug LY reduced the number of intromissions preceding ejaculation. In Exp. 2, 4 or 2 ug SKF did not affect copulation, but 20 ug SKF reduced the number of ejaculations. In Exp. 3, co-administration of 1 ug LY + 2 ug SKF did not summate to facilitate behavior; both LY alone and 10 ug SKF did not facilitate copulation, while 40 ug SKF did reduce copulation. In Exp. 4, 10 ug Flup failed to block the reduction in intromissions preceding ejaculation caused by 1 or 10 ug LY.

In summary, the D1 agonist LY163502 decreased the number of intromissions preceding ejaculation (ejacula threshold). This effect was not enhanced by co-administration of the D2 agonist SKF83256, nor blocked by co-administration of the DA antagonist cis-flupenthixol.

324.10 INTERACTION OF D1 AND D2 RECEPTORS IN THE EXPRESSION OF SENSOMOTOR DEFICITS IN MPTP-TREATED MICE. J. J. Wiesmuller*, D. Fine, K. Bajberg, and N. K. Swain, Depts. of Psychology and Pharmacology, The Ohio State University, Columbus, OH 43210.

MPTP is a useful tool for understanding the etiology and pharmacology of parkinsonism. We recently reported that MPTP-treated mice exhibit motor impairments and sensory neglect after small doses (0.2 mg/kg) of haloperidol that have no effect in control animals. To determine the relative contribution of dopaminergic D1 and D2 receptors to this effect we examined the ability of selective D1 or D2 agonists, SCG 23390 (D1) and l-sulpiride (D2), to induce sensorimotor deficits. Small to moderate doses of SCG 23390 (0.2-0.5 mg/kg) induced only motor deficits and only in MPTP-treated mice; whereas a higher dose (1.5 mg/kg) caused both motor and sensory impairments in both groups of mice. MPTP-treated mice were supersensitive to the motor, but not to the sensory, effects of haloperidol (0.2 mg/kg) and l-sulpiride (50 mg/kg) induced pronounced sensorimotor deficits in control, but not saline-treated mice. These findings suggest that D1 and D2 receptors contribute to different aspects of sensorimotor behavior and their interaction is necessary for the expression of deficits in DA-depleted mice.
MONOAMINES AND BEHAVIOR IV

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omitted from the ACS F, the depression by adenosine was adenosine and N-(9H-fluoren-9-ylmethyl) adenosine, selective D2 receptor antagonists. Supported by the Science & Engineering Research Council.

324.11

FACILITATION OF SEXUAL AROUSAL IN MALES AND FEMALE RHESUS MONKEYS


324.12


324.13

THE EFFECTS OF SELECTIVE DOPAMINE D1 OR D2 RECEPTOR ANTAGONISTS ON THE ESTABLISHMENT OF ROOD-INDUCED PLACE CONDITIONING. J. E. Hoffer and J. J. Benjamin. Dept. Psychol., Queen's University, Kingston, Ontario, K7L 3N6 Canada.

324.14

DOPAMINE D1 AND D2 ANTAGONISTS DIFFERENTIALLY BLOCK CONDITIONED LOCUTION BASED ON BEHAVIORAL, D1 OR D2 AGONISTS. R. J. Benjamin and J. J. Benjamin. Dept. Psychol., Queen's University, Kingston, Ontario, K7L 3N6 Canada.

325.1

ELECTROPHYSIOLOGICAL EVIDENCE THAT ADENOSINE REQUIRES MAGNETISM FOR ITS INTERACTION AT THE D1 ADENOSINE RECEPTOR J.C. Birks* and T. N. Stone. Department of Physiology, St. George's Hospital Medical School, London SW1 7RE, UK.

325.2


PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

324.11

Dopamine agonists have long been reported to facilitate male sexual activity in humans (Abel, S.A. & Schiller, Pharmacology 16:177, 1963) as well as in rats. The clearest demonstration of this action has been made recently with a new compound, LY163502 (Eli Lilly & Co.), which is highly potent and selective for the dopamine D2 receptor. It facilitates mating in both male and female rats at very low doses (Foreman & Hall, Psychopharmacology 86:43, 1983; Vancs Toth 86:413, 1987). Male and female rhens were tested with this drug in a confined partner paradigm, in which the normal sexual stimulus was allowed to be replaced by a standardized stimulant animal of the opposite sex but not to contact it physically. The test was carried out for a 30 min sexual session, such as erection or presentation, as well as other behaviors, were scored in 10 sec blocks. At doses of 10 µg/kg LY163502 caused a reliable increase in sex controls in the erection rate of male animals (p<0.001). A dose of 1 µg/kg was ineffective. Penile (a passive contact signal), grooms and feeding were also elevated at this dose, but only at 25 µg/kg. The increases in erection and penile activity, in contrast to those in feeding and grooming solicits, were completely dependent on the presence of a female stimulus animal. Males that had been castrated at birth were also tested in this procedure, at a dose of 25 µg/kg. These animals had received repeated testosterone injections at various times, but all treatments ceased at least one year before the present experiment. The castrates responded behaviorally much as the intact males had, but with substantially lower erection rate and quality (on a 1 to 3 scale). Female rats, tested with a mate stimulus animal, also displayed increased sexual behaviors when treated with 10 µg/kg (25 µg/kg) adenosine (5OµM) occasionally gave a larger increase in potential size than could be rapidly reversed by theophylline (50µM). This response could also be elicited by N-ethylcarboxamidoadenosine, 2-phenylamino-adenosine and N-fluoro-N-ethyladenosine, selective A2 receptor agonists. Supported by the Science & Engineering Research Council.
325.3
EVIDENCE THAT THE DEPRESSION OF RECURRENT INHIBITION FOLLOWING TETANIC STIMULATION IN THE RAT HIPPOCAMPUSS IS MEDIATED BY GABA RECEPTORS. B.L. Morel, A. C. Brugnon, and W. A. Wilson. Deps. of Pharmacology, Physiology, Pediatrics (Neurology) and Medicine (Neurology), Duke University and Veterans Administration Medical Centers, Durham, NC 27710.

A variety of mechanisms have been proposed to explain the depression of synaptic inhibition which follows repetitive firing. We have shown that GABA receptor antagonists (pentobarbital, picrotoxin) reduce tetanic depression of excitatory responses to the entorhinal cortex (EVT) in the rat dentate gyrus by an action on the inhibitory interneurons (West and Bragdon, 1987).

A similar effect has been demonstrated in cultured hippocampal neurons (Harrison et al., 1988). We have further reported that tetanic depression is due to a decrease in the amplitude of the inhibitory postsynaptic potential (IPSP) in the rat dentate gyrus, and that the GABA receptor antagonist picrotoxin can prevent this depression.

We have also shown that muscimol (a GABA receptor agonist) can restore tetaniC depression of excitatory responses to the entorhinal cortex (EVT) in the rat dentate gyrus, and that the GABA receptor antagonist picrotoxin can prevent this depression.

These results suggest that GABA released during repetitive firing can suppress GABA-mediated effects acting at GABA receptors on interneurons.

Supported by NIH grants MH37084, MH 23770, NH 77771, and VA.

325.5
ARGITOXIN-636 BLOCKS GLUTAMATE-MEDIATED SYNAPTIC TRANSMISSION ON EXCITATORY RESPONSES TO EXCITATORY GLUTAMATE IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. C.L. Cox, J.H. Ashe, and A.C. Collins. Inst. for Behav. Genetics, Univ. of Colorado, Boulder, CO 80309.

These drug effects were only slightly reversed with over one hour of washout.

In prepubertal neurons from rats, the actions of hyper- polarization and increased EPSPs may be relevant to the use of amphetamines in the attention deficit syndrome.

Supported by the Hospital for Sick Children Foundation and MRC.

325.4
PHARMACOLOGICAL CHARACTERIZATION OF ELECTROPHYSIOLOGICAL EFFECTS OF NICOTINE IN MOUSE HIPPOCAMPUS. R. R. Fournier, A. J. Kugelshaffer, and A. C. Collins. Inst. for Behav. Genetics, Univ. of Colorado, Boulder, CO 80309.

Previous studies have indicated that nicotine induces a concentration-dependent increase in the hippocampal CA1 population spike (PS) and the induction of secondary spiking. In an attempt to characterize the receptor(s) which are responsible for these effects, a series of nicotinic and muscarinic antagonists were tested for their effects on CA1 PS's from DMA slices. The following drugs were tested: picrotoxin (PSTZ; 10-100 kM) increased the PS and induced secondary PS's. Two nicotinic antagonists, but none of the muscarinic antagonists (D-TC; 10-100 kM) had little or no effect on CA1 PS's. Several of these antagonists have been tested for the ability to inhibit effects of nicotine, whereas Hex was not.

Preliminary data indicate that D-TC is also ineffective for blocking responses to nicotine. These results suggest that the pharmacology of nicotinic receptors in brain is different from that found in the periphery.

(Supported by R. J. Reynolds Tobacco Co.)

325.6
EFFECT OF DESIPRAMINE AND AMPHETAMINE ON NORADRENALINE SYNAPTIC TRANSMISSION IN VIVO STUDIES IN THE RAT DORSAL HIPPOCAMPUS. O. Curet and C. de Montigny. Dept. of Psychiatry, McGill University, Montreal, Canada H3A 1A1.

This study was undertaken to determine the effects of desipramine (DAM/AM) on noradrenergic (NE) synaptic transmission, CA1 hippocampus pyramidal neurons were recorded with five-barreled microelectrodes. The central barrel was filled with NaCl and side barrels with NE (0.05M in 0.2M NaCl; pH 4), acetylcholine (0.02M in 0.2M NaCl; pH 4) and 2M NaCl. A bipolar stimulating electrode was positioned in the locus coeruleus (LC). 150 square pulses were delivered at 1 Hz with an intensity of 800 µA. The degree of suppression of pyramidal neurons firing activity was quantified from peristimulus time histograms.

DM (0.5-5 mg/kg, i. v.) and AMHP (0.25-5 mg/kg, i.v.) both decreased the effect of the LC stimulation and increase the duration of the response of the same neurons to the microiontophoretic application of NE. The subsequent intravenous injection of lithium, an α-adrenergic antagonist, restored the effectiveness of the LC stimulators.

It is concluded that the acute administration of either DM or AMHP, by increasing the concentration of NE in the synaptic cleft, results in a decreased effectiveness of the LC stimulation and an increased activation of terminal α-adrenergic autoreceptors.

325.8
ANTIPIELEPTIFORM EFFECTS OF CYCLIC AMP IN THE CA3 REGION OF RAT HIPPOCAMPAL SLICES. S. A. Reker* and P. J. Leher (SPON). P. F. Porzrodo. Program in Neuroscience and Section of Neurophysiology, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

We have observed that cyclic AMP (CAMP 5-80µM) produces a reversible decrease in frequency of electrophysiologically recorded interictal discharges induced by bethanecol (40µM) and theophylline (10-20µM) in the CA3 region of rat hippocampal slices. Since it has also been shown that cyclic AMP, an α-adrenergic agonist, and its analogs produce a similar effect, we have tested the hypothesis that CAMP acts indirectly by being converted extracellularly to adrenaline by two enzymatic steps catalysed by a phosphodiesterase (PD E ) and a 5'-nucleotidase (5NT). This hypothesis predicts the following: 1) blocking the breakdown of CAMP would attenuate its effects and 2) nonhydrolyzable analogs of CAMP would have minimal or no effects. In contrast to the first prediction, the effect of CAMP is potentiated by coapplication of a β-methylenedependence 5'-phosphate (AMP-CP 8-16µM) or 3-isothioulyl-adenosine (IBMX 10-25µM), a SNT and a PDE inhibitor (with an additional adenosine receptor blocking action) respectively. Control experiments with these compounds suggest that they do not, by themselves, have antiepileptiform effects. Further, two hydrolyzable derivatives of CAMP, dibutyryl (10-20µM) and ethylamino-cAMP (10-10µM) are as potent as CAMP in their discharge suppressor action. These data are not consistent with the idea that the antiepileptiform action of exogenously applied CAMP is mediated solely by metabolic conversion to adrenaline and suggest an additional possiblity a direct mode of action involving extracellular sites for CAMP and its first metabolite product, 5'AMP.

(Supported by USAMRDC contract DAMD17-86-C-4029, AFOSR85-0178 and NIH grant NS11530)
EFFECTS OF AN ANESTHETIC ALPHA-2 ADRENERGIC AGONIST, DEXMEDETOMIDE, ON PHA synaptic transmission in hippocampal slices. V.A. Doze, M.B. MacVicar, M. Maze, J.J. Kendig (SPON: H. Schulman), Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Dexmedetomidine, a highly specific alpha-2 adrenergic receptor agonist, produces a behavioral state analogous to general anesthesia in the rat. The anesthetic effect is stereospecific and blocked by alpha-2 blocking agents. The present study examined electrophysiological responses to dexmedetomidine in a rat hippocampal slice preparation.

* Sympathetically evoked field potentials were recorded from CA1 neurons by standard extracellular techniques. The application of dexmedetomidine (100 nM) produced reversible concentration-dependent increases in both population spike (PS) and field EPSP amplitudes (110-200% of controls). Higher concentrations of dexmedetomidine blocked both PS and EPSP. This apparent biphasic response differs from the responses (depression of PS and EPSP) observed with volatile general anesthetics in this preparation, but resembles to some extent the effects produced by pentobarbital.

*Supported by NIH Grant NS11082 to JJK.


The action of CNQX, a purported quisqualate antagonist (Hontone et al. 1987, Soc. Neurosci. Abst.), was studied on hippocampal slices in vitro. Bath application of CNQX (2-4 uM) rapidly and reversibly blocked the Schaffer collateral and mossy fibre evoked responses. The blockade of CNQX was not associated with changes in membrane potential, membrane resistance or spike accommodation. In addition, the fast and slow GABA mediated inhibitions were not altered by CNQX. CNQX (2-3uM) also blocked spontaneous and evoked bursts in the CA3 region induced by NMDA and kainate, as well as the plateau phase of the kainate evoked burst with 0.5uM CNQX. The effects of CNQX were also tested on the currents induced by excitatory amino acids in the presence of TTX using the single electrode voltage clamp technique. inward currents induced by quisqualate (10uM), kainate (200nM) and NMDLA (200uM) were reduced to 32.4%, 78.17 and 62.11% of control respectively by 10uM CNQX.

From our observations we suggest that the transmitter for the e.p.s.p. evoked by Schaffer collateral and mossy fibre stimulation acts on the quisqualate type receptor and furthermore that this same receptor plays a substantial role in burst generation.

R.S.N. received support from INSERM and MRC(C). M.G. was a fellow of the Fondation du France.

325.12 DEPRESSION OF SYNAPTIC TRANSMISSION BY -CONOTOXIN IN THE RAT HIPPOCAMPAL SLICE. D.B. Foley* and A.P. Feldman, Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G 1Y6.

The release of neurotransmitters from the presynaptic terminal of a neuron is dependent on calcium ions that enter via channels in the plasma membrane. -conotoxin GVIA (-CTX) is an irreversible inhibitor of synaptic transmission in a number of peripheral synapses, and it binds to synaptic plaques in brain. The effect of -CTX has been observed in both N and L-type calcium channel channels where having no lasting effect on the T-type channel, suggesting that the N- and/or L-channels are responsible for regulation of synaptic transmission. We studied the effect of -CTX on synaptic transmission in the in vitro hippocampal slice preparation at both the Schaffer collateral (SC)-CA1 and mossy fibre (MF)-CA3 synapses. Transverse rat hippocampal slices 400 µm thick were maintained in artificial CSF (aCSF) at 24-25°C in an interface chamber. Population excitatory postsynaptic potentials (eEPSPs) were recorded either in stratum pyramidale or in stratum lucidum of CA1. -CTX (3uM) was applied as a microdrop to the surface of either stratum radiatum of CA1 or stratum lucidum of CA3. Application of 3uM -CTX to the dendritic field of CA1 or CA3 irreversibly decreased SC evoked eEPSPs 30-50% (N=3) and MF evoked eEPSPs 35±4% (N=6), respectively, indicating a net decrease in synaptic transmission. Microdrop application of control vehicle had no effect on synaptic transmission at either the SC-CA1 (n=3) or MF-CA3 (n=5) synapses. The decrease in synaptic transmission in the hippocampus upon application of -CTX to the major excitatory inputs of both the CA1 and CA3 regions supports involvement of the N- and/or L-type calcium channel currents in the regulation of synaptic transmission at these sites. Depression of synaptic transmission by -CTX was less than 50%, however, which may indicate a role for the T-type or a non -CTX-sensitive calcium channel subtype in synaptic transmission. Nonuniform exposure of synaptic terminals to -CTX could also be a factor in the magnitude of the changes observed. Modulation of the properties of these channels may alter the efficacy of synaptic transmission and may be involved in the mechanism of long-term potentiation. (NIH grants NS11535 & HL51164 and AFOSR 85-0178).

325.13 NMDA CHANNEL ACTIVATION MEDIATES PROLONGED PAIRED PULSE DEPRESSION OF POPULATION SPIKE AMPLITUDE IN RAT HIPPOCAMPUS. R.S. Goldman and C.F. Stevens. Section of Molecular Neurobiology and Department of Neurology, Yale University School of Medicine, New Haven, CT 06510.

The effect of varying magnesium (Mg) concentrations on the population spikes of paired pulses was studied in area CA1 of rat hippocampal slices. Reduction in Mg concentration resulted in a dose dependent depression of the ratio of the spike amplitude of the second pulse to that of the first pulse. This reduction lasted for hundreds of milliseconds, and was not influenced by bicuculline, implying that this effect was not related to an effect of Mg on inhibition. Across different Mg concentrations there was a correlation between the amount of quisquulate induced currents and bursts in comparable extent as the population spike, implying that the paired pulse depression was due primarily to a change in the excitability of the postsynaptic cell. Evoked responses following a spontaneous firing showed a similar inhibition.

In terms of possible mechanisms, we favor the view that prolonged depression is due to Mg entering the postsynaptic terminal and by low Mg results in prolonged sodium channel inactivation. However, we cannot exclude the possibility that transmission of the synaptic current to the soma is impaired due to shunting of current by activated channels in the dendrites.

325.14 DO GENERAL ANESTHETICS HYPERPOLARIZE MAMMALIAN CNS NEURONS? J.J. Kendig and M.B. MacVicar, Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Unitary theories of anesthesia propose that all general anesthetics share a common mechanism. One suggested common action is hyperpolarization of CNS neurons through increase in potassium conductances. The present study examined whether inhalation anesthetics (halothane, enflurane, isoflurane, methoxyflurane) share this action. Synaptically evoked responses, resting potential and conductance were measured in the area CA1 of neurons of rat hippocampal slices. Agents were administered via the surface gas stream at equivalent anesthetic concentrations (1 MAC). Only halothane produced hyperpolarization (3.5 mV) accompanied by a conductance increase (10-15%). Isoflurane depolarized CA1 neurons and decreased the extracellular potassium conductance, but produced biphasic (hyperpolarizing/depolarizing) actions. All agents blocked the synaptically evoked population spike but were variably effective in depressing EPSP amplitude in the order methoxyflurane > isoflurane ≥ enflurane ≥ halothane. These results do not support a unitary theory of anesthesia; instead, there appear to be agent-specific actions at multiple membrane sites. Protocol approved by Stanford Laboratory Animal Care Panel. Supported by NIH Grant NS11082 to JJK.
SYNAPTICALLY EVOKED SPIKE INITIATION IN DENDRITES OF CA 1 HIPPOCAMPAL NEURONS. H.B. Maciver and J.J. Kendig. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Both dendritic and somatic sites have been proposed as the spike initiation zone in synthetically activated hippocampal CA 1 neurons. The present study used fixed and moving extracellular electrodes (K acetate filled, 0.5-1.2 M KCl) and intracellular electrodes (K acetate filled, 80-120 M KCl) in rat hippocampal slice. A bipolar stimulating stimulus was placed in s. radiatum. Field potentials were monitored at several levels along the axis of CA 1 neuron apical dendrites. At low stimulus intensities a negativity, corresponding to the positive EPSP recorded in the somatic region, reached maximum amplitude at the level of the activated fibers in s. radiatum. At stimulus intensities which produced a half maximal population spike population, a second larger but briefer negativity was superimposed on the first, with maximum amplitude 150-200 µV closer to the somatic layer, but still well within the dendritic layer in s. radiatum. This second wave occurred 0.5 to 2 ms before the population spike at the somatic level or the action potential recorded intracellularly in the soma. These results support a dendritic site for synthetically evoked spike initiation in apical dendrites of CA 1 neurons. *Protocol approved by Stanford Laboratory Animal Care Panel. Supported by NIH Grant NS13108 to JJK.


The influences of alcohol on 1) agonist binding to muscarinic acetylcholine receptors and 2) receptor coupling to guanine nucleotide-dependent transducer proteins (G proteins) were determined in membranes prepared from rat brainstem. The effects of ethanol on carbachol binding (determined in competition studies with [3H]methylyscopolamine) at temperature dependent: AC 37°C the majority of receptors were in a high affinity conformation and ethanol increased agonist affinity; at 37°C the majority of receptors were in a low affinity conformation and ethanol further decreased binding affinity. At 23°C, ethanol inhibited the binding of 2 nM [3H]N-methylscopolamine to high affinity receptors (Kd = 40 ± 15 pmol/L). Guanine nucleotide sensitivity of the [3H]Doxa-N binding (an indication of receptor interaction with G proteins) was greatly increased by ethanol (Kd = 60 ± 22 pmol/L). Guanine nucleotide sensitivity was also decreased by a number of other alcohols with the following order of potency: n-octanol>butanol>ethanol>isopropanol>methanol. In this action, alcohol resembled anesthetic (Biochem. Pharmac. 26:1201, 1987), raising the possibility that ifereence with receptor-G protein coupling underlies depression of synaptic transmission caused by a variety of agents. Supported by GM-37948 and AA-0768.


We have demonstrated that anesthetics interfere with the interactions of muscarinic acetylcholine receptors with transducer G proteins in both brain and heart (e.g., Biochem. Pharmac. 26:1201, 1987). We studied the influence of halothane on the muscarinic receptor-mediated stimulation of GTPase activity by G proteins in membranes isolated from rat striatum and cerebral cortex. Membranes were incubated with [3H]GDP[ta] for 1-10 min at 37°C in a regenerating buffer containing 2 mM GTP. Halothane-induced GTPase activity was 26 ± 15 pmol, P(0.05) released/mg protein/min in the striatum. ACCh stimulated the low Kd GTPase activity by 40-80%, with an EC50 of 5 ± 1 μM and a maximal effect with 10-20 μM ACCh. Pretreatment of the membranes with pertussis toxin under ADP-riboseylating conditions decreased both basal and receptor-stimulated GTPase activity by up to 60%. Equilibration of the membrane with halothane (0.1-4%) resulted in a decrease in ACCh-stimulated, but not basal, GTPase activity. The IC50 for this action was about 0.5X. These findings suggest that halothane selectively interferes with receptor-G protein coupling. (Supported by GM-37948, AA-0768 and the Georgia Heart Association.)

Ethanol (5-100 mM) potentiated the effect of GABA on 36Cl-infux; while at concentrations ≥ 50 mM ethanol activated Cl channels directly. The effect of ethanol was specific for GABA, receptor-gated Cl channels, since ethanol did not potentiate glycine-induced 36Cl-infux in the same neurons. Ethanol (20 mM) which does not exhibit a direct effect, increased the value of GABA from 11.4 ± 0.8 mV to 4.8 ± 0.5 mV without affecting the maximal response. Both the enhancing and direct effects of ethanol were blocked by GABA antagonists such as bicuculline, picrotoxinin, and inverse agonists of the benzodiazepine site such as RO1-4513 and FG-7142. Ethanol potentiated the effect of GABA-induced 36Cl-infux was also reversed by DMCM. The effects of the inverse agonists were blocked by the benzodiazepine receptor antagonist Ro15-1788. Both Ro15-4513 and FG-7142 reversed direct and GABA potentiating effects of ethanol at concentrations lower than those that exhibit inverse agonistic activity in the 36Cl-infux assay in cultured neurons. These results suggest that ethanol facilitation of GABAergic events involves GABA receptor-gated Cl channels. The mechanism of interaction may be responsible for some of the pharmacological effects of ethanol. Supported by NIAAA grant AA04090.


Clinical and behavioral studies have shown that tolerance develops to many of the therapeutic actions of benzodiazepines, however, the mechanisms responsible for the development of tolerance to benzodiazepines is unknown. Recent studies in our laboratory (Wilson and Gallager, Eur. J. Pharmacol., 136:333, 1987) have found regional differences in the effects of chronic diazepam treatment. Using diazepam-inflated silastic implants to maintain pharmacologically relevant brain concentrations of diazepam, it was observed that rats exposed to diazepam for 3 weeks show a decreased responsiveness of dorsal raphe neurons to iontophoretically applied GABA. In addition, potency of pentylenetetrazol for elicitation, however, the same treatment failed to alter GABA sensitivity. Using the same sustained release chronic treatment protocol, we have measured GABA-stimulated 36Cl-infux into brain membrane vesicles from rats treated chronically with diazepam or vehicle. To investigate possible regional differences in response to chronic diazepam exposure, we have analyzed vesicles prepared from cortex and cerebellum. Cortical membrane preparations from chronic diazepam-treated rats exhibited a decreased GABAergic stimulation of 36Cl-infux. In contrast, chronic diazepam treatment had no effect on GABA-stimulated 36Cl-infux in cerebellar membrane preparations. These results support the suggestion that chronic benzodiazepine treatment results in a reduction in GABA/BZ receptor function in some, but not all, brain regions.


We have previously demonstrated GABA sub-sensitivity in dorsal raphe neurons (DRN) following chronic BZ agonist exposure. Our prior studies analyzed in vivo sensitivity of DRN to iontophoretically applied GABA in anesthetized rats following chronic silastic-implanted silastic capsule treatment. To determine whether GABA sub-sensitivity was dependent upon dorad raphe affinities, we analyzed GABA responses of DRN neurons in brain slices obtained from rats exposed to diazepam-filled silastic capsules for 3 weeks. Midbrain slices from vehicle and diazepam-treated rats were maintained simultaneously under the same in vitro conditions. Artificial cerebrospinal fluid containing 2.5 mM phenylephrine was used to induce pacemaker-like activity. Firing rates of DRN in control and diazepam-treated slices were comparable (19±2 vs 18±2 spikes/10 sec). Indicating that sensitivity to phenylephrine was not altered by chronic diazepam exposure. DRN neurons in slices from chronic diazepam-treated rats had reduced responses to iontophoretically applied GABA, when compared to the GABA sensitivity observed in control slices (Table 1). Furthermore, following chronic diazepam exposure, sub-sensitivity to GABA is observed in DRN neurons both in vivo and in vitro. This suggests that the decreased GABAergic responses induced by chronic diazepam exposure reflect changes intrinsic to the dorsal raphe nucleus.

326.5 MODULATION OF GABA-MEDIATED DEPOLARIZING SYNAPTIC RESPONSES BY NMDA IN IMMATURE HIPPOCAMPAL NEURONS. R. Corredetti*, J.L. Gaiares*, Y. V. BenAri and E. Cherubini. (BNPON - R. Amedei. INSERM U-29, 123 Bd Port-Royal, Paris 14, FRANCE)

Intracellular recordings were made from immature CA3 rat hippocampal neurons (0-8 days) in the in vitro slice preparation. In adult CA3 pyramidal neurons, stimulation of the inhibitory afferents evoked a depolarizing potential which was mediated by GABA acting on GABA A receptors. In CA1 and CA3 B neurons, the response was mediated by GABA acting on GABA A receptors. In newborn rats, stimulation of the same region evoked a long-lasting (400 ms) depolarizing potential which was blocked by GABA acting on GABA A receptors since it was blocked by GABA (0.01-10 uM). The reversal potential of the response (-20 to 4 mV; n=9 with K Cl and -53 to 4 mV; n=8; mean ± SEM with K-acetate-electrodes) was very close to the reversal of the response to exogenous applied GABA (0.01-10 uM with K Cl and -53 mV with K Cl and -41 mV, respectively). These results suggest that the depolarizing potential is due to a GABA A receptors. In newborn rats, stimulation of the same region evoked a long-lasting (400 ms) depolarizing potential which was mediated by GABA acting on GABA A receptors. In adult rat cortical neurons, 326.6 INHIBITION OF GABA-STIMULATED CHLORIDE INFUX INTO MEMBRANE VESICLES FROM RATS CEREBRAL CORTEX BY ANTIDEPRESSANTS AND NEURONOTICS. M. kodaka, E. Molinoff, K.F. Figueroa and H.J. Yamamura. Dept. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724 and Nathan Kline Inst. for Psychiatric Research, Orangeburg, NY 10962.

One of the important side-effects produced by clinically used antidepressants and neuroleptics is convulsive seizures. The convulsive effects of antidepressants and neuroleptics are dose-dependent, but the convulsive effects is not known. To elucidate the convulsive effects of antidepressants and neuroleptics, we examined the effects of amphetamine, SKF 10810, meprobamate, (-)mirtazapine, chlorpromazine, haloperidol, pimozide and spiperone on GABA-stimulated 36Cl-infux into membrane vesicles from rat cerebral cortex. All of these membrane vesicles were preincubated with test compounds for 10 min. 36Cl-infux was determined by the addition of GABA to the tubes containing the membrane vesicles. After incubation for 3 sec, the uptake was terminated by the addition of ice cold buffer followed by rapid vacuum filtration through Whatman GF/C filters. Amphetamine, meprobamate and (-)mirtazapine inhibited GABA-stimulated 36Cl-infux uptake at 1uM, 44.1±4.8%, 30.4±4.9% and 49.1±0.8% (Kodaka et al., 1986). These results showed inhibition of GABA-stimulated 36Cl-infux uptake at 10uM and 100uM concentration. These findings suggest that the convulsive and sedative effects are related to the GABA-activated chloride influx.
326.7

**B-CEE, GCS8516 AND RO55-1788: A COMPARISON OF ACTIVITY IN MOUSE AND RAT.** L.S. Labinsky and J.L. Vaupel. Janssen Research Foundation, Spring House, PA 19477

In both mouse and rat, B-CEE, GCS8516 and RO55-1788 selectively enhance the anticonvulsant activity of diazepam (but not phenoobarbital) in a metrazol seizure test. However, in the mouse, B-CEE and GCS8516 are also potent proconvulsants. In the rat, B-CEE and GCS8516 at doses ≤ 5 mg/kg i.p. and GCS8516 at doses < 1 mg/kg i.p. but not RO55-1788, potentiate convulsant activity. In electrically-induced seizures: GCS8516 > 0.8 (0.4-1.6) mg/kg i.p.; B-CEE = 3.8 (1.8-7.6) mg/kg i.p. Their proconvulsant duration on the mouse, GCS8516 and B-CEE are more potent proconvulsants than they are BZD antagonists. Unlike B-CEE (C50-9 mg/kg i.p.) or DMC (C50 = 13 mg/kg i.p.), neither B-CEE (=100 mg/kg) nor GCS8516 (=20 mg/kg) causes convulsions. In the rat, however, B-CEE produces weak proconvulsant activity while GCS8516 is practically devoid of proconvulsant effects in either chemical or electrical-induced convulsions. Thus, in the rat, these agents are more BZD antagonist-like than proconvulsant. These data suggest: 1) as previously reported, B-CEE is more than a pure BZD antagonist, 2) that GCS8516 and B-CEE are similar in their effects on seizure threshold and their relative potency is consistent i.p. and s.c. and 3) that potential differences in the pharmacological characteristics of the GABA/BZD complex exist between mouse and rat.

326.9

**STRUCTURE ACTIVITY RELATIONSHIPS FOR STEROID POTENTIATION OF GABA RECEPTOR-INDUCED CHLORIDERE UPTAKE IN THE DEVELOPING CORTICAL CORTEX.** A.L. Morrow, R.L. H. Funk* and S.M. Paul*.

Cortical synaptosomes were prepared from cerebral cortices of adult male SD rats. Muscimol-stimulated 36Cl uptake was measured for 5 seconds in the presence or absence of various steroids (1 µM). THDOC potentiated the effect of muscimol (3.0 µM) by 1.8±1.5 nmole/mg protein. 10-3-21 acetate had similar activity increasing muscimol-stimulated 36Cl uptake by 12.2±1.1 nmole/mg protein. 3-α-0H,5-α-DHP and 3-α-0H,21-21 acetate were also inactive. Other inactive derivatives were 3-α-0H,5-α-DHP, 3-α-0H and 21-21 acetate. The glucocorticoids and mineralocorticoids were inactive at concentrations between 0.2 and 5 µM. These data define the specificity for steroid hormone metabolite activity on GABA-mediated chloride ion flux in the rat brain.

326.11

**ONTOGENY OF GABA-STIMULATED CHLORIDE UPTAKE AND ITS ENHANCEMENT BY DIAZEPAM IN BRAIN SYNAPTONEUROSOMES**. C. Kellogg and G. L. Pledger. DEPT. OF PSYCHOLOGY, UNIV. OF ROCHESTER, ROCHESTER, NY 14627.

Late gestational exposure to diazepam (DZ) has been shown to induce selective and lasting alterations in the activity (Kung & Brain Res., Vol. 75, 1979). Ligated binding assays have indicated the presence of DZ at appropriate receptor sites in fetal brain in the form of DZ-like components. In the present investigation, DZ exposure during the latter half of gestation was examined in synaptoneurosome preparations of whole brain at 20 and 21 days of gestation and of cerebral cortices from 7 to 59 days of age. DZ-stimulated CT uptake was detectable from 20 days of gestation with an increase for GABA-activated uptake from 20 to 21 days gestation (p < 0.01). Maximal stimulation of uptake during this period occurred at 50-100 µM GABA and was 4.76±0.36 nmole/mg protein at 20 days and 6.13 ± 0.12 nmole/mg protein at 21 days. DZ decreased the Bmax for GABA stimulation by 38% (a magnitude similar to that observed in adult tissue) without changing the maximal stimulation of the Bmax. Following the above Protocol (Bmax for GABA stimulation was similar to adult values (Bmax) at 7 and 14 days, but DZ induced a considerably greater shift (50%) in the Bmax at these ages than observed in fetal or adult tissue. At 21 days, the Bmax for GABA-stimulation increased to 11.25 µM, but the effect of DZ was still pronounced. By 28 days postnatal age, the Bmax and the response to DZ appeared adult-like. The maximal stimulation increased throughout development reaching peak values (19 ng/mg protein/10 sec) by day 21. These results indicate that DZ is capable of altering an effector response by its action in the rat fetus. The developmental shifts in the Bmax for GABA stimulation of CT uptake and in the responsivity to DZ suggest underlying changes in the polyribosomal complexes containing the DZ and GABA recognition sites and the CT isomerophore. Supported by grant no. NS31890.

326.12

**DIBUTYL CAMP AND PORKOKLIN DECREASE GABA RECEPTOR-MEDIATED CI UPTAKE IN RAT BRAIN SYNAPTONEUROSOMES.** Gunter Schneider* and Rochelle D. Schwartz. Dept. Pharmacology, Duke Univ. Medical Center, Durham, NC.

The functional activity of the GABA receptor-coupled Cl channel was measured under CaM-dependent phosphorylation conditions. Pretreatment of rat cerebral cortical synaptoneuroosomes (10 min, 30°C) with dibutyl cAMP (BiCAMP, 0.1-3.0 µM) produced a concentration-dependent decrease of muscimol-induced 36Cl uptake (5.2 ± 1.3 - 55.5 ± 2.3%, BiCAMP (1 µM) decreased the maximal effect 3.3 ± 4.0%) but not the potency of muscimol to stimulate 36Cl uptake. Porgokin (FSK, 30-200 µM) also inhibited muscimol-induced 36Cl uptake (16.3 ± 1.5 - 42.0 ± 3.9%). The inhibitory effect of FSK (20 µM) was potentiated by 2 µM preincubation. The same time course was observed for FSK-induced CaM generation in the intact synaptoneuroosomes. However, the inactive FSK analog, 1,6-dideoxy forskolin (20-200 µM), similarly inhibited the muscimol response (19.3 ± 4.5 - 67.5 ± 5.1%), indicating the effect of FSK might also involve mechanisms unrelated to activation of adeny cyclase. The inhibition of the muscimol response by both BiCAMP (1 µM) and FSK (50 µM) was antagonized by 10 µM and 12.2 ± 0.9 µM, respectively. These data suggest that CaM-dependent phosphorylation mechanisms regulate the activity of the GABA receptor-coupled Cl.

Supported by NIH grant NS 24577 and PMAF Award to RDS.
Amino Acids: GABA and Benzodiazepines V

326.13


It is assumed that the main effect of benzodiazepines (BZD) is potentiation of y-aminobutyric acid (GABA) action through the interaction mechanism, and that the co-solubility of GABA suggests rapid penetration through the plasma membrane and responses induced intracellularly. In this study, we compared the effects of RO2-07-013 (FLU) and RO2-07-012 (FLU-0213) on dorsal root ganglion cells which were isolated from rats (250-350g) anesthetized with urethane/alpha-chloroethane. RO2-07-013 is a quaternary derivative of FLU, and would not be expected to pass across the membrane (Gaza, S. & Farb, D. H., J. Neurosci., 6:1857, 1986). FLU and RO2-07-012 showed similar potential responses on GABA-depolarization. Trayskin-taking (7990/mU) abolished the GABA action and also the BZD effects, while electrical membrane properties were not affected. These results assure that BZD binding sites related to these effects are exposed at the extracellular surface. Augmentation of the membrane resistance by FLU was long-lasting, whereas that by RO2-013 disappeared just after termination of its action. The effects of RO2-07-012 but not FLU were abolished by trypsin. FLU preferentially depressed the steady-state outward currents, whereas RO2-07-012 modified an earlier phase of outward currents as well. These results suggest existence of intracellular loci of action of BZD as well as extracellular binding sites.

326.15

THE REVERSAL POTENTIAL FOR GABAERGIC CURRENT IS INFLUENCED BY MEMBRANE VOLTAGE. J.A. Bird and P.B. Medrano. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Recordings made in slices of visual cortex (Scharfman and Sarvey, Neurosci. 23:407-422, 1987) show that GABA is hyperpolarizing when applied to cell bodies and depolarizing when applied to axonal fibers. We attempted to study this regional difference in chemosensitivity by applying GABA to the somata and processes of visual cortica neurones maintained in culture. The occipital cortex of embryonic and postnatal rat brain were dissected and plated onto a lawn of neuronal cells and astrocytes in a medium containing Earle’s balanced salt solution, and antibiotics to which the neuronal cells are insensitive. Cultures maintained for 1 to 6 weeks at L-1539, with 5% rat serum. With standard whole-cell, patch-clamp techniques, recordings were made from cells bathed in a solution of 140 mM K gluconate, 1.1 mM EGTA, 10 mM Hepes/K OH, 2 mM MgCl2, 2 NaCl, 2 NaATP, 0.2 NaGTP; 1 glucose. GABA, 50-100  ìM, was applied by pressure from pipettes with 1 mm diameter of 2-4 mm positioned near the cell surface. The reversal potential for GABA applied either at the soma or at distances of 50 to 150 um along dendrites was similar and within 5 mV of the holding level when cells were depolarized by kainic acid but not by 500 ìM baclofen. Our results suggest that synaptic transmission involving GABA receptors can be blocked by a variety of factors that affect the membrane voltage. In this paper, we show that the reversal potential for GABA is influenced by the membrane voltage, and that the reversal potential is influenced by the membrane voltage.

326.16


There is indirect evidence that a tonically active inhibitory GABAergic network withdraws in the PAG, the PAG plays a significant role in the activation of the PAG-mediated analgesia. We examined the effect of GABA, picrotoxin (PTX) and bicuculline methiodide (BICM) on baseline activity of PAG cells in PAG slices as well as in anesthetized rats. Recordings were made from neurons in PAG slices that were perfused with normal or calcium-free or CaC-dextrose solution (KRS). Drugs were applied next to the recording cell by perforation in pressure . In the in vivo experiments, drugs were applied by ionophoresis or pressure injection to neurons in choral hydrate anesthetized 250-300 gram rats.

Response of 50 cells in PAG slices and 16 cells in intact animals were examined. Injection of GABA produced inhibition in 87% of PAG cells and its effect could be blocked by BICM and PTX. Sensitization of response to GABA was noted in 60% of the cells. BICM increased the baseline firing rate in 62% of the cells. Application of BICM caused multiple spiking of PAG cells in both types of preparations. Neurons in all regions of the PAG were responsive to GABA and BICM and no localization of specific effect was observed. This study was supported by NIH-NINDS grant #NS02643.

326.17

GABA-EVOKED RESPONSES OF MYELINATED DORSAL AND VENTRAL ROOT FIBERS: MODULATION BY K+ CHANNEL BLOCKERS. E. Liivak and H.E. Morrisey, Department of Pharmacology, University of Toronto, Toronto MS5 1A8.

GABA (y-aminobutyric acid) and THP (4,5,6,7-tetrahydro-2-benzoxazinyl-3-one) release Ca2+ from intracellular stores. In intact dorsal root fibers of isolated bullfrog sciatic nerve—the effect is best observed by reducing Ca2+ in the extracellular fluid (Van der Putten, J. Neurosci. Methods, 4:329-342, 1981). Cells in these slices exhibited typical properties for CA5 or CA2 hippocampal pyramidal neurons. Most cultures demonstrated a high incidence of small, fast PSPs; EIPSP's could trigger action potentials at a rate of 0-5/sec. These cultures also produced spontaneous slow (up to 1 sec. duration) IPSPs. Stimulation of the stratum radiatum produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition. The spontaneous IPSPs produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition. The spontaneous IPSPs produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition. The spontaneous IPSPs produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition. The spontaneous IPSPs produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition. The spontaneous IPSPs produced an IPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of inhibition.
326.19

(-)-BACLOFEN HAS A DUAL MECHANISM OF ACTION IN RAT SPINAL HORN IN VITRO. P R Boden and R G Hill. Parke-Davis Res Unit, Addenbrookes Hospital Site, Hills Rd, Cambridge, CB2 0QH, UK.

In dorsal root ganglia, the GABA agonist (-)-baco

lofen depressed inward calcium currents and reduced evoked release (Robinson, B and Taylor W R, Br J Pharmac 89:661, 1986) while in hippocampus (Newberry, M R and Nicoll, R A, Nature 310:879, 1984) it is given an outward potassium current. We have examined the action of (-)-baco

lofen on deep dorsal horn neurones by intracellular recordings from animals of 9-16 day old rat spinal cord. On all neurones studies (-)-baco

lofen (100M=30M) had a post-

synaptic hyperpolarising action and reduced apparent input resistance. This response was blocked by intracellular Cs+ and extracellular Ba++. Indeed Ba++ itself produced additional synaptic activity which was depressed by both (-)-baco

lofen and 200M mg. (-)-baco

lofen therefore in spinal dorsal horn both post-synaptically increases potassium conductance and pre-synaptically depresses calcium (barium) currents.

326.20

PHARMACOLOGY OF INHIBITION IN THE GLOMERULAR LAYER OF THE OLFACTORY BULB. W L Mitchell, M E Shively and H J Duncan. Dept. Anat. & Cell Biol., Univ. Coll. Med., Cincinnati, OH 45267. Two different central GABA receptors have been identified: GABA_A receptors are activated by muscimol, and blocked by picrotoxin and bicuculline; GABA_B receptors are activated by baclofen, but not muscimol and are not blocked by picrotoxin or bicuculline. A recent autoradiographic study (Bower et al., 1987) demonstrated a striking segregation in the olfactory bulb of the rat: GABA_A receptors are present in all layers of the bulb; GABA_B receptors are almost completely limited to the glomerular layer. We have studied the properties of GABA_A and GABA_B synapses in the mammalian olfactory bulb (OB).

Orthodromic responses were recorded with micropipettes in the glomerular layer of the OB. With electrical stimulation of the olfactory nerve (ON) using a stimulus of 25 to 200 msec, the magnitude of the second response decreases at shorter intervals. Both baclofen and muscimol inhibited the response to both pulses. Inhibition of the second response was not blocked by picrotoxin.

These results suggest that inhibition is mediated by both GABA_A receptors and GABA_B receptors in the glomerular layer. GABA_B receptors mediate classic postsynaptic inhibition; GABA_A receptors may be the source of GABA-mediated presynaptic inhibition. Staining with GAD antisera suggest that there are large and small GABA terminals in the glomerular layer while there are only small terminals in the infra-glomerular layer. These two classes of terminals may be related to GABA_A and GABA_B synaptic sites. (Supported by: NS23548, US Army DAMD 76-6-C-6005, WU NS 25223.)

327.1

GMl GANGLIOSIDE PROTECTS LOSS OF PLASMA MEMBRANE FUNCTION AFTER CEREBRAL ISCHEMIA. C G Wacker, V B Bharucha, S E Karpiak and S P Mahadik. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry, State University of New York, Upstate Medical University, Syracuse, NY 13210. We have reported that ganglioside GM1 treatment protects acute injury processes (tonic imbalance, edema, loss of membrane function) and delayed neuronal death (Mg-ATPase) indicative of plasma membrane failure after global ischemia. To understand the molecular mechanisms of these protective effects we have used a reproducible model of focal ischemia. Ischemia was produced by combined permanent medial cerebral & common carotid artery occlusion (MCAo+CCAo) with 1 hr temporary contralateral CCAo in rat. Levels of Na,K-ATPase, Mg-ATPase and tissue ions (Na+, K+ & Ca++) in ischemic tissue (primary and peri-infarct cortical areas) were compared with the contralateral side in rats treated with saline or GMI. In the primary infarct area 72 hrs after ischemia levels of Na,K-ATPase & Mg-ATPase in rats were reduced by 45% & 37% respectively in the saline rats, and both by only 15% in GMI treated rats. In the peri-infarct areas losses in both enzyme levels in saline treated rats were minimal (~15%), with almost no losses in GMI treated rats. The tissue levels of Na+ & K+ paralleled the loss of Na,K-ATPase in saline treated animals but the levels of Ca2+ increased slowly, reaching a maximum after 72hrs.

327.2

IN VITRO PROTECTION AGAINST CEREBRAL HYPOXIA BY LOCAL ANESTHETICS. C A Cushman, L F Leslie, M W Smith, R J McRae, M J Fajardo, C S Donn and A Schurr. Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292. Numerous studies in recent years on potential antipyrine drugs and their pharmacology, where many of them employed in vivo models of cerebral ischemia/hypoxia. For example, selective activation of such drugs we have been using the in vitro rat hippocampal slice preparation. In a recent study we demonstrated the depression effects of lidocaine on synaptic function using this in vitro system. If such depression is the result of metabolic arrest or ion fluxes attenuation, then it is likely that local anesthetics should exhibit antipyrine properties.

Rat hippocampal slices were incubated with non-depressive doses of either lidocaine, 2-chloroprocaine or cocaine 60 min prior to their exposure to 15 min hypoxia. The rate of recovery of synaptic function (evoked population spike) following the hypoxic episode was used as an index of hypoxic tolerance. Slices treated with 0.1 mM of any of the three local anesthetics exhibited a significant increase in the recovery rate of synaptic function from hypoxia as compared to control untreated slices.

The antipyrine properties of local anesthetics may stem from their ability to reduce Na2K influx (and possibly its concomitant Ca2+ influx), or from conservation of ATP via metabolic slow down- or both.

327.3

FREE RADICAL SCAVENGERS PROTECT AGAINST PEROXIDATIVE DAMAGE IN THE HIPPOCAMPAL SLICE. T C Pellman, K L Neel and M L Moss, Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814. Hydrogen peroxide decreases synaptic efficacy and impairs postsynaptic spike generation (Pelplan, Brain Res. 364:37). Since the damage in part is free radical generation, the free radical scavengers dimethylsulfosiloxane (DMSO) and thiourea would be expected to afford some protection.

Slices of hippocampus were prepared from brains of euthanized guinea pigs. Stimulating electrodes were positioned in the stratum radiatum to stimulate afferents to CA1 region. Recording electrodes were positioned to record the population spike and the synaptic potential selectively. Input-output curves were generated by varying the stimulus intensity between 0 to 2.0 mA. DMSO or thiourea was applied for 30 min prior to exposure to hydrogen peroxide. The responses in control were compared to the responses after 30 min in 0.005% peroxide plus scavenger.

These results show that protection of higher doses of thiourea were not as effective as they directly decreased the population spike. DMSO (50 mM = 0.36%) had no direct effects but protected postsynaptic spike generation from peroxide damage. Impairment of synaptic efficacy by peroxide was still present in DMSO. (Supported by: NINDS NS 25223.)

327.4

ISCHEMIC TOLERANCE IS INCREASED IN THE CITERNAL INFUSION MODEL OF RAISED INTRACRANIAL PRESSURE: POSSIBLE ROLE OF THE EXTRACELLULAR MICROENVIRONMENT IN THE PREVENTION OF EXCITOTOXIC DEGENERATION. A G Duniga and D T Ross, Department of Clinical Neurosciences, Brown University and Department of Neurosurgery, Rhode Island Hospital, Providence, RI 02902. Elevation of the intracranial pressure above the mean arterial pressure has been shown to temporarily stop cerebral blood flow. In order to study the effects of raised intracranial pressure on the development of cerebral ischemia, a cerebroebutional infusion model was used to elevate intracranial pressure above mean arterial pressure for various intervals. Male Long Evans rats were anesthetized with chloral hydrate and xylazine, orally intubated, and artificially ventilated with room air. The left common carotid artery was cannulated for continuous measurement of arterial blood pressure. Two 25-gauge needles were inserted into the right jugular vein for measurement of intracranial pressure and the other for instillation of antifibrinolytic fluid. Intracranial pressure was raised above blood pressure for five to thirty minutes by osmotic instillation of artificial CSF at ~100 mOsm. The animals were allowed to survive for one to seven days and the hippocampal formation was examined for ischemic change in 32 micron Nissl stained sections by light microscopy.

No significant behavioral or histological changes were seen unless the duration of ischemia was between 25-30 minutes, when animals survived for up to seven days. The osmolar infusion of large volumes (10-50 c.c.) of artificial CSF may prevent the accumulation of extracellular potassium and excitotoxic amino acids which occur in ischemia. As a result it appears that pathological and behavioral changes occur only after periods of ischemia significantly longer than those required to produce these changes in vessel occlusion models. Studies are in progress to determine the extracellular concentration of excitatory amino acids during raised intracranial pressure.

CEREBRAL ISCHEMIA III
CEREBRAL ISCHEMIA III

WEDNESDAY PM

327.5 NOREPINEPHRINE IN THE GERILIC HIPPOCAMPUS: EFFECT OF DSP-4 ON PYRAMIDAL CELL LOSS AFTER TRANSIENT ISCHEMIA. K. Nishio*, J. K. Morse, C-S. Lin and J. N. Davis (SPON: F. Elderidge). V.A. Medical Center and Duke University Medical Center, Durham, NC 27710.

Hippocampal pyramidal cells are vulnerable to transient periods of ischemia in the Mongolian Gerbil and the rat. We measured the levels of norepinephrine (NE), the diaminergic neurotransmitter tyrosine hydroxylase-immunoreactive fibers and the effect of DSP4 pretreatment on pyramidal cell counts in ischemic Gerbils. The NE levels were similar in the dentate gyrus and the septal, medial and temporal portions of the hippocampal gyrus. Dopamine was barely detectable. The laminar distribution of fibers was similar to the rat, but the fibers were present in the dentate gyrus and more were seen in stratum oriens of CA1. Animals were pretreated with 100 mg/kg of DSP4 14 days before undergoing 5 minutes of bilateral carotid artery occlusion and sacrificed 7 days later. There was significant worsening of pyramidal cell loss in the CA1, CA3, hippocampal regions of the DSP4-pretreated animals compared to saline-pretreated sham operated animals (surviving neurons were 11 vs. 96, 71 vs. 49%, and 80 vs. 66%, respectively; repeated measures ANOVA, p=0.0001). Coronal biopsies from the treated animals before sacrifice for cell counting showed that DSP4 treatment had lowered cortical NE (Saline vs. DSP4, 0.29 ±0.02, 0.10 ±0.03; p=0.0001, *t* test). Our data are consistent with previous reports in rats suggesting that NE neurons may modulate neuronal death in hippocampal pyramidal cells. (Supported by the VA and NS 06233)


Protective effects of two new drugs against ischemic brain injury were examined using a rat focal ischemia model. They are charybdotoxin (CTX: purified from a scorpion venom), which is a specific inhibitor of the Ca2+ activating channel, and an algameric derivative of prostaglandin E1 (MR-356).

The focal ischemic lesion was produced by permanent ligations of the middle cerebral artery and the common carotid artery (CCA). CTX (0.15 mg/kg) or MR-356 (66 mg/kg) was administered 30 min before 50 min after the induction of ischemic insult. Three days after the focal ischemia, the formation of brain edema and the changes in the serum creatinine (Na, K and Ca) were measured. Motor deficits and memory disturbances were also evaluated by motor performance tests (which included inclined plane, balance beam and prehensile tests) and the massive avoidance task, respectively.

The brain edema and tonic denervation were reduced in the pre-ischemic treatment with CTX or in the post-ischemic treatment with MR-356. Motor deficits and memory disturbances were ameliorated in accordance with the reduction of brain edema. Possible protective mechanisms of these new drugs in brain ischemia will be discussed.

327.9 (S)-EMOPIAML, A NEW CALCIUM ANTAGONIST OF THE VERAPAMIL GROUP. S. Gell, H. Gsell, E. Agrumi, L. Biagini and A. Tiscia. SINUSIA, Pisa, Italy.

Experimental studies have yielded conflicting results concerning the therapeutic effect of calcium antagonists in different models of the rat focal cerebral ischemia. This work is related to the fact that several compounds with well-documented cardiovascular effect on the heart or the central nervous system are available to reach the brain in sufficient quantities. (S)-emopamil (6-(methylphenoxy)-1-phenyl-2-(2-pyridyloxy)ethyl1-hydrochloride) is a recently developed intravenous calcium antagonist available for parenteral use that crosses the blood-brain barrier easily. Based on our measurements of radioactivity after intravenous administration of (S)-emopamil to rats, the relative cerebral concentration of (S)-emopamil was 6.1 to 8.2 times higher than that of verapamil. (S)-emopamil was found to prolong the survival of mice in a dose-dependent fashion. In rats, (S)-emopamil decreased cerebral blood flow in brain ischemia models whereas verapamil showed only marginal effects in these models. Apart from its calcium antagonistic properties, (S)-emopamil has affinity to the high affinity receptor in the SHKETANIN binding test (kI = 4.9 mmol/L). In measurement of the neuronal damage in ischemic rats, verapamil showed 70% more damage than that of verapamil. The new compound was also more effective in the prevention of injury in the ischemic rat rats. (S)-emopamil was found to be more effective in the prevention of injury in the ischemic rat rats. (S)-emopamil was found to be more effective in the prevention of injury in the ischemic rat rats.


Monosialoglucosilolipid (GM1) treatment of animals has consistently shown to ameliorate outcome following a variety of CNS injury models including cerebral ischemia. We here report the effects of the inner ester derivative of the ganglioside following transient 4 vessel occlusion in adult rats (Ponseti and the Biphasic Stroke 10:267, 1979). The ganglioside was systemically administered at a dose of 20 mg/kg and its efficacy was evaluated by i) monitoring of the cortical EEG and ii) assessment of the degree of morphological damage in the CA area of the hippocampus. Preliminary data indicate that the ganglioside improves EEG recovery during the first 2 weeks following the ischemic episode. In addition, the ganglioside increases the percentage of animals showing less severe hippocampal CA neuronal loss. These effects may possibly be related to the ganglioside prevention of glutamate neurotoxicity observed in vitro (Feronow, M. J., et al., Stroke 1988; Skager, S.D. et al., this meeting). To further validate such a hypothesis, the ganglioside capability to decrease post-ischemic seizure threshold in vivo may be evaluated, as well as the ganglioside capability to ameliorate delayed hypoxic neuronal damage in rats currently being evaluated in gerbils following transient bilateral common carotid artery occlusion.
**CEREBRAL ISCHEMIA III**

**327.11**

**MK 801 MODULATES METABOLIC RESPONSE TO PERINATAL ASHYXIA**

S. R. Zarnegar*, Division of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305

**327.12**

**EFFECTS OF LY178002 AND LYS256548 ON ISCHEMIA-INDUCED BRAIN DAMAGE. J. A. Clemens, M. L. Phillips, M. B. Roush, and J. A. Panetta.** The Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285.

**327.13**

**DECREASED DELAYED TEMPORAL FUSION METER RESPONSES FOLLOWING TRANSIENT GLOBAL CEREBRAL ISCHEMIA**


**327.14**

**PRETREATMENT WITH THE NMDA-ANTAGONIST DEXTRORPHAN ATTENUATES NEURAL DAMAGE AND EDema IN FOCAL CEREBRAL ISCHEMIA IN THE RABBIT**

J. Saleh, G.K. Steinberg*, J. Roush* and J. A. Panetta*. The Lilly Research Labs, Indianapolis, IN 46285.

**328.1**

**CORTICAL AND MEDIANCABLE CONNECTIONS OF THE INTERMEDIODORSAL NUCLEUS IN THE RABBIT**

T. W. D'Agostino. Biological Anthropology, Harvard University, Cambridge, MA 02138.

The mediiodorsal nucleus (MD) is the principle thalamic source of projections to the frontal cortex. Subunits within MD may have been shown to project to discrete subregions of prefrontal cortex in monkey, cat and rat from distinct midbrain areas. However, the afferent and efferent connections of the intermediodorsal nucleus (IMD) on the midline between the MD nucleus has not been characterized. We have investigated the fine structure of WGA-HRP by micropipette were identified in MD in a series of rats brains and the tissue was processed with TEM. In a number of cases cortex and hippocampus overlapping the MD have been shown to project to the MD nucleus were removed by cautery to prevent uptake of tracer that might confound IMD labeled connections. Injections of the tracer were made in the total cortex. Histological measurements of ischemic neuronal damage and edema when administered before the onset of focal cerebral ischemia (Goldberg, M.P. et al, Neurosci Lett 80:11, 1987). We studied the efficacy of systemic pretreatment with DX in our rabbit model. These drugs may have therapeutic potential in the treatment of cerebral ischemia.

**328.2**

**ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS**

J. A. Panetta*. Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305.

The mediodorsal nucleus of the thalamus (MD) is the principle thalamic relay to the prefrontal cortex. Using GABA(immunohistochemistry) and glutamate (gamma-Glu-Glu, astrocytes, provided by J.E. Maizell, the present study examined the organization and morphology of GABA and glutamate as well as the MD nucleus in the adult primate MD, with the aim of providing information on the local circuit neurons and projection neurons of this nucleus. GABA and glutamate-like neurons were found to differ on several features of their morphology and distribution. These data may have therapeutic potential in the treatment of cerebral ischemia. 

**328.3**

**ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS**

J. A. Panetta*. Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305.

The mediodorsal nucleus of the thalamus (MD) is the principle thalamic relay to the prefrontal cortex. Using GABA(immunohistochemistry) and glutamate (gamma-Glu-Glu, astrocytes, provided by J.E. Maizell, the present study examined the organization and morphology of GABA and glutamate as well as the MD nucleus in the adult primate MD, with the aim of providing information on the local circuit neurons and projection neurons of this nucleus. GABA and glutamate-like neurons were found to differ on several features of their morphology and distribution. These data may have therapeutic potential in the treatment of cerebral ischemia.
328.3
DIFFERENCES IN THE ORGANIZATION OF GRANULAR FRONTAL ASSOCIATION CORTEX OF MACAQUE, STUMP-TAILED MACAQUE, AND HUMAN.
S. L. Goldman-Rakic, Sec. Neuroanatomy, Yale Sch. of Med., New Haven, CT 06510.

The connections of granular frontal cortex in the prosimate primate Galago sarcoptes were investigated by injecting WGA-HRP or [3H] amino acids into frontal and posterior association cortex of 15 hemispheres. Cyto- and myeloarchitecture (using the Gallyas myelin method) were also examined. Galago organization was compared to that of macaque monkeys (arthropod primates).

Galagos and macaques share many features of granular frontal organization, including strong connections with posterior parietal cortex. Macaques have two tiers of parietal-receptive areas, an arcuate (Walker's areas 8a, 8b, 45) and a periparietal (“dorsalocortex”) tier (9, 46, 12). Galagos possess arcuate-like cortex only (Goldman-Rakic, Brain Res. 142:1044, '86), but lack features indicative of periparietal areas: (1) individual posterior parietal areas have fewer zones of termination in galago granular frontal cortex than in macaques. (2) The AV and AM thalamic nuclei, which project to principal cortical areas, are weakly labeled in galagos (light label in AM, little or none in AV). (3) Galago parietal-receptive cortex does not project densely to the deep orca or central gray, structures which receive strong projections from macaque dorsolateral areas. (4) The insula projections only lightly to galago granular frontal cortex; macaque areas 45 and 12 have strong insular connections. (5) Several distinctively myelinated principal zones, e.g., the very light cortex of the ventral bank of the principal sulcus (area 46), are absent in galagos. We interpret these differences as evidence that prosimian primates (such as Galago) are lacking some or all of the dorsal-lateral areas found in anthropoid ("higher") primates and that these areas are evolutionary vestiges. Our results suggest there are qualitative differences among primates in "prefrontal" organization.

328.5
ORIGINS OF AFFERENTS TO PREFRONTAL CORTEX IN THE RAT.

In order to better understand the role of the rat’s prefrontal cortex (PFC), retrograde transport of fluorescent tracers was used to define the distribution ofafferent fibers from the whole brain. A mixture of yellow and/or true blue was injected (0.1-0.3 ul) into one or two areas of the PFC, ipsi- or contralaterally. After 4-7 days of survival, perfusion and histological processing were done according to Audinat, Conde & Crepel. (Exp. Brain Res. 69:439-443, '85). Different PFC areas received projections from different parts of the mediadorsals of the thalamus (MD) according to Krstek & Price (J. Comp. Neurol. 171:157-192, '77), although areas of overlap exist in ventral MD project to both insular and prelimbic area (PL). Moreover a band-like organization of thalamo-cortical relations is apparent in the PFC. PFC zones can be distinguished on the basis of their afferents: CAI field of hippocampus and n. paratenialis (plp) project massively to PL whereas n.galatinus and primary olfactory cortex project to insular cortex. These results support the hypothesis that the role of insular cortex is to integrate multisensory information while that of medial PFC is to analyze information prior to motor acts.

328.7
FUNCTIONAL HETEROGENEITY OF THE RAT PREFRONTAL CORTEX (PFC).
M. Rasmussen, B. Barnes and P. S. E. McNaughton. (SPO: D. Adel Afifi). Department of Anatomy, University of Iowa, Iowa City, IA 52242.

Previous studies have shown that the rat ventromedial (VM) thalamic nucleus sends projections to both the infralimbic (IL) and lateral agranular (AG) cortices. To determine whether these projections involve thalamo-cortical, we conducted a retrograde double labeling experiment with biocytin and fast blue injected in these cortical areas. Injections in AG strongly labeled neurons in the ventroposterolateral (VPL), posterior nuclear group, and VM. Somewhat more lightly labeled were neurons in the zona incerta (ZI) and rhomboid nuclei (Rh). Additionally, cells in the nucleus reuniens were very sparsely labeled. Injections in IL labeled neurons in the RC, Rh, and paraventricular nuclei, and usually (but not always) neurons in the mediodorsal nucleus and ZI. Neurons in the anterior thalamic nuclei were never labeled by infralimbic injections. Double labeled neurons were observed very rarely in the Re and Rh.

The lack of labeling in VM neurons following IL injections was particularly surprising. The discrepancy between our results and Herkenham's ('84) autoradiographic study may be due to a lack of volume of the Re and Rh. In all cases we observed ventral to VM, uptake of tracer by these cells probably explains the labeling seen in IL in previous studies.

The lack of collateralization among thalamic neurons innervating the infralimbic and lateral agranular cortex suggests that the modulation by the thalamus of these two cortical areas is largely separate. The limited collateralization observed in Re and Rh is consistent with the idea (Jones '85) that these nuclei are involved in diffuse cortical activation.

328.8
SPATIALLY SELECTIVE DISCHARGE OF VISION AND MOVEMENT MODULATED POSTERIOR PARietAL NEURONS IN THE RAT.
R. L. Crut and B. S. McNaughton. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Functional properties of neurons of the posterior parietal cortex (Krieg, 1946, J. Comp. Neurol. 84, 221; Kobli et al., 1987, Brain Res. Rev., 23, 127) in freely moving rats were studied in a spatially working memory task on a radial arm maze, using the stereotrode recording technique (McNaughton, Crut et al., 1983, J. Neurosci. Meth. 3, 391). Most of the 145 cells studied exhibited selectivity for a particular state of motion (e.g., left-turns, right-turns, straight running, still). Visual and response characteristics were tested by manipulating the room light source. 24% (31/128) of neurons tested were visually responsive, exhibiting either phasic excitation or inhibition with changes in room illumination or preferentially discharging while the animal was at a particular orientation relative to the light source. Some visually responsive cells showed location preference for the location of hippocampal "place" cells (O'Keefe, 1979, Prog. Neurobiol. 13, 419).

At least part of this location specificity appeared to be generated by an interaction between somatosensory and visual inputs. Cells maintained a spatial firing bias when the light source was extinguished but showed a shift in bias corresponding to the movement of a visual stimulus. We conclude that some cells in the posterior parietal cortex could be involved in spatial representation (Supported by NS30331 to BLW).
ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

328.13


We have investigated the distribution of Choline acetyltransferase immunoactivity in neuron cell bodies (CAT-IR) and Gamma amino butyric acid immunoactivity (GABA-IR) in the rat frontal cortex. This paper describes the two-dimensional distribution of CAT-IR and GABA-IR in adult and aging rats. In the anterior frontal cortex (area 11, 12, 13 and 25) the density of CAT-IR and GABA-IR was higher in the aging rats compared to the adult rats. In layer II-VI, the density of CAT-IR and GABA-IR was lower in the aging rats than in the adult rats.

328.14

THE MULTIMODAL ASSOCIATION CORTEX (ANTERIOR LATERAL, PERICRANII AND MEDIAL SUPERSWAREN ASSOCIATION) IS NOT ESSENTIAL FOR LEARNING AND MAINTAINING THIS INSTRUMENTAL CONDITIONING OF THE EYE BLINK IN CATS. Supported by USPHS grants HD000158 and HD25400.

328.9


Monocular antibody Cat-301 recognizes a surface antigen on certain CNS neurons. It appears to label intercrossed areas in the thalamic and cerebral cortex (Hendry et al., 1986) and the magnocellular visual pathway (DeVoe et al., Soc. Neurosci. Abstr., 12:130, 1986). We have examined the distribution of Cat-301 immunoreactive neurons in regions of the primary posterior parietal and frontal cortices which are strongly and topographically interconnected.

In both, the distribution is characteristic for each cytoarchitectural area, but varies greatly between areas, with abrupt changes at their borders. In posterior parietal cortex (PPC), the posterior intraparietal (PI) area (area 7), mediadorsal sector (MD) and ventral parietal area (VPA) (area 5b) are topographically interrelated. In the motor cortex (motor area 4a), labeling is stronger in the supplementary motor and premotor cortex (area 6), and in the caudal prefrontal areas (area 8a and 8b). Two patterns of neuronal distribution are evident: throughout PPC, and in the heavily-labeled frontal areas, immunopositive cells are concentrated in lower layer III and in layer V, and approximately 15-25% are pyramidal, the remainder being non-pyramidal. In contrast, in the more rostral prefrontal areas (which are highly-stained), labeled cells are diffusely distributed across layers II-VI, and almost all are non-pyramidal.

The laminar distribution of immunopositive cells in heavily-labeled areas matches that of the association neurons known to project between parietal and frontal cortices, and each heavily-labeled frontal area is known to be connected (but not exclusively connected) with a heavily-labeled area in PPC. This suggests that the differential distribution of Cat-301 IR in these regions may be related to their interconnectivity.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX V

WEDNESDAY PM


Two and possibly three subdivisions of visual cortex in the region of the visual hemifield were evoked by low levels of stimulation (10-50µ amps), and artificially flattened, sectioned parallel to the surface, and reacted for HRP each animal) were injected into medial and lateral aspects of the medulla is also observed bilaterally with slight origins and distribution of afferent neurons in the newborn and adult monkeys, and marmosets. In all of these primates, injections of WGA-HRP were made into cortical regions that included the primary sensorimotor cortex, involving areas 4, S1, and 5. Labeling was also found in area 7b of the macaque but not in the cat. In order to investigate the extent of cortical projections in the macaque and cat that might terminate in the HGN, multiple injections (0.5µl each) of the sensitive anterograde tracer WGA-HRP were made into cortical regions that included the primary sensorimotor cortex and in laminae II to V in premotor cortex (area 6), medial and lateral aspects of the primary somatosensory cortex (S1), area 5, 7a and also in the limbic cortex. Soma populations were observed as overlapping or segregated in primary sensory motor cortex and in laminae II to V in premotor cortex (area 6). The present investigation confirms previous degeneration studies which identified a direct cortico-hypoglossal projection in the macaque but not in the cat. In addition the present study demonstrates in the macaque a bilateral projection to the reticular region adjacent to the HGN. The axons of this projection may turn medially to terminate within the HGN or may directly synapse upon ventrally and laterally directed dendrites of HGN motoneurons.


Degeneration studies have demonstrated a direct projection from the tongue area of primary motor cortex to the hypoglossal nucleus (HGN) in the macaque but not in the cat. In order to investigate the extent of cortical projections in the macaque and cat that might terminate in the HGN, multiple injections (0.5µl each) of the sensitive anterograde tracer WGA-HRP were made into cortical regions that included the primary sensorimotor tongue area.

Following injections in the macaque and cat, labeled axons were observed in the HGN. The axons that reached the HGN did so by way of the pyramidal tract, originating in lamina V and four day old infants.

- The present investigation confirms previous degeneration studies which identified a direct cortico-hypoglossal projection in the macaque but not in the cat.
- In addition, the present study demonstrates in the macaque a bilateral projection to the reticular region adjacent to the HGN. The axons of this projection may turn medially to terminate within the HGN or may directly synapse upon ventrally and laterally directed dendrites of HGN motoneurons.

AXON-COLLATERALS OF PYRAMIDAL CELLS IN LAMINA III AND V OF AREA 4 OF MONKEY'S CORTEX, REVEALED BY INTRACELLULAR HRP. R. Porter, S. Ghosh and R.E.W. Fyffe Experimental Neurology, John Curtin School of Medical Research, Canberra, 2001, Australia.

Lamina V neurons of monkey's motor cortex with axons in the pyramid tract have been stained by intracellular injection of HRP (Hamada et al., Neurosci. Lett., 22: 233-236, 1981). Six lamina V and four lamina I pyramidal neurons in area 4 of M. fascicularis were completely reconstructed after intracellular HRP injections. The lamina III pyramidal neurons differed from those in lamina V: they lacked long basal dendrites. Although axon collateral arbors of lamina III pyramidal cells varied in the extent of their distribution, 3 to 12 collaterals arose from each axon. The largest lamina III intracortical axon collateral branch sent to laminae and extended for at least 3mm. The lamina I pyramidal neurons with axons collateral arbors of lamina V pyramidal neurons, which consistently exhibited three to five collaterals. Although the longer of these could extend for more than 3mm, the branches of lamina V axon collaterals were confined to laminae V and VI, whether or not the lamina V neurons were demonstrated to be PNs.


Experimental and clinical studies have suggested the presence of a motor-related field within the depth and the lower bank of the anterior cingulate sulcus. Indeed, corticospinal projections arise from this cortex. Despite this, little is known about the connections of this motor field, and in particular, its relationship to other cortical motor representations. Thus, a study was undertaken to examine the ipsilateral somatotopical distribution of cingulate afferents to MI and MII. Rhesus and cynomologus monkeys were injected with retrograde tracers fast blue, diamidino yellow and HRP into face, forelimb, and hindlimb representations of MI and MII. Some injections were made to both MI and MII were identified in the cortex of the lower bank and fundus of the cingulate sulcus. Labeling was spatially separate from area 4 (MI) and area 6 (MII) on the basis of the mainly somatotopical representations within MI and MII arise from common cingulate regions, with those projecting to face located rostrally, hindlimb caudally and forelimb between. Afferents to MI originated primarily from layer V while those to MII originated from both layers I and V, indicating that MI and MII are each part of a disproportionately organized set of motor fields. Labeling with retrograde tracers and fluorescent tracers for three and four days. Results support the fact that a spatially separate and somatotopically organized motor field resides within the cingulate cortex that has direct projections to both MI and MII. Its location and relation to the motor cortices on one hand, and limbic cortices on the other, suggest an interplay between basic drives such as motivation and cortical motor mechanisms. (Supported by NS 14943.)
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX V

MOTOR ACTIVITY IN THE RAT. D. Asdourian, O. Hnatczuk.

The effects of bilateral electrical stimulation of the frontal cortex (Cx) on motor activity in albino rats show that the electrically driven output from the two hemispheres interact in ways specific to the relative intensities of stimulation of the two sides and, in some cases, to the sequence of Cx stimulation. Motor activity was recorded between two trapezoidal paras cervicales which is concerned with shoulder and forelimb movement. After the thresholds for driving activity were established between the paras cervicales, the rats were stimulated with subthreshold current or with suprathreshold current and the effects on the behavior were noted. The animals were divided into two groups: (1) When both Cx were stimulated simultaneously, a high-frequency response was observed. (2) When one Cx was stimulated with a suprathreshold current and the other with subthreshold current, no response was observed. (3) When both Cx were stimulated with subthreshold current but stimulation was presented successively rather than simultaneously, activity was driven only from the Cx that was first stimulated. This last effect was seen in 1/3 of our animals.

EXCITABILITY OF CORTICOSPINAL NEURONS DURING TONIC MUSCLE CONTRACTIONS IN MAN AND THE RAT.

A magnetic stimulus applied to the human scalp over the motor cortex causes a short latency contraction of contralateral limb muscles. This is presumed to result from the indirect excitation of corticospinal neurons with monosynaptic connections to motoneurons. The excitability of these cortical neurons can be estimated from the magnitude of the postsynaptic potentials produced by stimulating the corticospinal motoneurons by magnetic stimulation. In man the characteristics of these postsynaptic potentials can be derived from the firing probability of single motor units. When a subject increases the level of a sustained voluntary contraction the excitability of the corticospinal motoneurones estimated in this way becomes less. We conclude that the additional synaptic input to motoneurones required for a stronger sustained voluntary contraction comes from fiber systems other than the population of fast corticospinal neurones activated by magnetic stimulation.


How does the motor cortex produce independent finger movements? Microelectrode evokes movements of a single digit (Asanuma & Rosen, 1972), but averaging has shown that single cortical neurones often influence more than one spinal motoneurone pool (Cheney & Fetz. 1972). To study further the processes that produce independent movements, motor area and primary motor cortex neurones are being examined in terms of their movement fields. Defined here as the subset of active movements with which a given neuron discharges.

Motor fields of motor cortex neurones can be classified as: single digit, if the neurone discharges with movement of only one digit in one direction; contiguous, if discharge occurs with movement of adjacent digits in the same direction; broad, if discharge occurs with movement of non-contiguous digits or with movements in opposite directions. Digits 1 (thumb), 2 and 5 have greater representations than digits 3 and 4 among neurones with single digit motor fields. But among contiguous field neurones, digit 3 is most often the field's best digit. No contiguous field neurones have been found with best digit 1 or 5. The premotor cortex, compared to the motor cortex, has relatively few neurones related to these finger movements, though all three motor field types have been observed. Support: K05-NS01150-03 to M.H. Schieber; ROI-NS17777 to W.T. Thach.


The supplementary motor area (SMA) is thought to play a role in the performance of movement. One hypothesis is that the SMA may influence the responsiveness of area 4 neurones to kinesthetic stimuli. Changes at the Control of Posture and Movement, 331-346, '73. Previously, we (Schieber, et al., to be published: Abstr. Vol. 19, 1987) reported that bilateral SMA cooling did not modify the kinesthetic responsiveness of area 4 neurones. In an additional animal, we have found that firing rates of some movement-related area 4 neurones are modified with SMA cooling. A rhinoceros monkey was trained to fix and extend the wrist in response to movement of a visual target on a visual monitor. The monkey's hands were attached to a molded form coupled to a torque motor which produced a simulated spring load. For juice rewards, the monkey was required to match a cursor as accurately as possible to a target. For a period of at least one second. Halfway through the random duration hold period, a 50 ms torque pulse was applied to perturb the wrist in either the flexion or extension direction. After training, the following items were implanted under percutaneous anesthesia and aseptic conditions: 1) Six bipolar EMG leads in the right forelimb muscles; 2) A recording chamber over the arm area of the conteralateral premotor cortex; 3) A cooling chamber placed within the sagittal fissure overlying the territory of the SMA; and 4) a heat resitant device.

Thus far, the firing patterns of 22 task-related area 4 neurones were analyzed during movements, hold phases and perturbations, before, during and after SMA cooling. 77% of these neurones changed their firing patterns during cooling. Premovement burst activity was reduced during SMA cooling while low activity during a hold phase was increased. No obvious modifications of the kinesthetic parameters of movement or torque perturbation responses were observed with cooling. Under the test conditions employed, cooling mildine structures including the SMA appears to modify the movement-related firing patterns of some area 4 neurones, but the consequences of this modification on motor performance are unclear.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX V

SAFETY STUDIES OF MAGNETIC AND ELECTRICAL TRANSRANSMISSION IN MAN. H.J. Kim, W. L. Levy, J. Do, D. Tucker. Departments of Neurological Surgery University of Miami School of Medicine, Miami, FL 33136, and University of Missouri-Columbia, Columbia, Missouri 65212.

A device for non-invasive transcranial stimulation has been introduced for the treatment of several disease conditions, including depression and Parkinson's disease. The safety of this method has been evaluated in ongoing studies from which we present our findings. A patient was treated with this method, and the results are presented in this paper. The device and its safety were evaluated using a controlled study. The results of the study showed that the device was safe and effective, and that it was well tolerated by the patients. This study was supported by the National Institutes of Health (NIH) grant NS02155 and NS22652.

SYNAPTONEBROSIS II


Cultured neonatal rat myotubes develop clusters of ACh receptors (AChR) where they adhere to the substrate. These clusters are often irregularly shaped, and their density in AChR-rich (AChR-Rich) muscle cells is significantly lower than in AChR-poor muscle cells. These AChR-poor muscle cells are more closely associated with the tissue culture substrate. In this study, we investigated the distribution of extracellular components at substrate-apposed AChR clusters in cultured rat myotubes. We found that extracellular components at substrate-apposed AChR clusters are organized into distinct domains that parallel the organization of the cluster. By 1 day of co-culture, the reaction product density of innervated cells was increased by 2-fold compared with uninnervated cells. Increases in synaptic current frequency seen at 4 days correlated with an additional 2-fold increase in synaptic current frequency. The early onset of synaptic transmission was reflected in increased reaction product density. The density of CO staining may be a direct indicator of the degree of early innervation of individual neurons. Supported by N02155, K08201, and N037062 (GM)

DISTRIBUTION OF ACH RECEPTORS, 43K PROTEIN AND Na* CHANNEL IN NEUROMUSCULAR JUNCTION AND CULTURED MYOTUBES. B.E. Fuchs* and M.P. Daniels. NHIB, NIH, Bethesda, MD 20892.

We have examined the distribution of ACh receptors (AChR), 43K protein, and Na+ channels in cultured rat myotubes and in the mature neuromuscular junction (NMJ). Using antibodies specific for AChR, 43K protein and Na+ channel, we have localized these components at the NMJ and in cultured myotubes. We found that AChR are localized at the NMJ and in the postsynaptic membrane of cultured myotubes. The distribution of 43K protein and Na+ channel is similar to that of AChR. These results suggest that the association of AChR, 43K protein and Na+ channel is a direct indicator of the degree of early innervation of individual neurons. Supported by NS22061, Klingenstein and Sloan Foundations (L.W.T), N ATO, FRM, & Philippe Foundation (RG) and NS07062 (GM).
330.5
THE PRESENCE OF A SYNAPTIC BASAL LAMINA ANTIGEN IN CULTURED MUSCLE CELLS OF XENOPUS L. M. Dahn and J. T. Landmesser. Dept. of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06268.

Activity blockade has been shown to rescue motoneurons and to increase the number of nerve side branches in the chick iliofibrariculus muscle. To assess the importance of synaptic interactions in controlling the growth of axonal side branches, we examined the temporal-spatial distribution of pre- and postsynaptic profiles in this muscle using MAb SV2 to stain synaptic vesicles and a polyclonal antibody to chick AChR clusters. Although muscle cultures in response to nerve stimulation or to Co2 stimulation or to Co2-29, co-localization of SV2 and MAb35 staining was not observed uninterrupted unbranched nerve trunk. AChR clusters appeared within several hours after nerve injury. Although clusters were located within diffusion distance of the nerve trunks, they were not co-localized to sites of SV2 staining, suggesting an early diffuse form of synaptic transmission. Co-localization was first observed following nerve side branch formation at the onset of cell death when SV2 became localized to side branches and ACh-R clusters near nerve trunks appeared and accumulated around side branches. The existence of co-localization, which was restricted to side branches, increased throughout the cell death period. A similar but earlier co-localization in cultured embryonic segments, this process is not activity dependent and may be required for motoneuron survival. Supported by NIH grant SR01 NS12940.

330.6
REGULATION OF SYNTHESIS OF THE 43kD POSTSYNAPTIC PROTEIN IN CHICK MYOTUBE CULTURES. J.W. Geddes, J. Chow, T. Hashab* and A.D. Grinnell. Jerry Lewis Neuromuscular Research Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

Direct cell-cell contact is important in synaptogenesis. There is evidence that this is enhanced by interaction between specific surface molecules, eg. neuronal cell adhesion molecule (N-CAM) and developmental regulation which influence the timing or probability of synaptogenesis. This study was directed to assay the effect of inhibition of N-CAM homophilic cell-cell adhesion on the initial establishment of neuromuscular transmission.

When anti-N-CAM (kindly supplied by Dr. U. Rutishauser) was added to co-cultures of nerve-muscle pairs, whereas 60-70% of the pairs were MPP-positive in non-inhibited control cultures, 20% of the cultures were MPP-negative. Analysis of the effects of anti-N-CAM revealed that only 40% of the identified cholinergic neurons released ACh upon muscle contact. These preliminary results suggest that N-CAM binding may be involved in the initial cellular recognition and initiation of contact-induced neurotransmitter release in this system.

(Supported by grants from MDA and NIH)

330.7

Activity blockade has been shown to rescue motoneurons and to increase the number of nerve side branches in the chick iliofibrariculus muscle. To assess the importance of synaptic interactions in controlling the growth of axonal side branches, we examined the temporal-spatial distribution of pre- and postsynaptic profiles in this muscle using MAb SV2 to stain synaptic vesicles and a polyclonal antibody to chick AChR clusters. Although muscle cultures in response to nerve stimulation or to Co2 stimulation or to Co2-29, co-localization of SV2 and MAb35 staining was not observed uninterrupted unbranched nerve trunk. AChR clusters appeared within several hours after nerve injury. Although clusters were located within diffusion distance of the nerve trunks, they were not co-localized to sites of SV2 staining, suggesting an early diffuse form of synaptic transmission. Co-localization was first observed following nerve side branch formation at the onset of cell death when SV2 became localized to side branches and ACh-R clusters near nerve trunks appeared and accumulated around side branches. The existence of co-localization, which was restricted to side branches, increased throughout the cell death period. A similar but earlier co-localization in cultured embryonic segments, this process is not activity dependent and may be required for motoneuron survival. Supported by NIH grant SR01 NS12940.

330.8

The reports by Fischbach (Dev.Biol. 28: 1972) and Bonner (Dev. Brain Res. 38: 1988) of electrical coupling between nerve-muscle contacts of cultured chick cells and that of Allen (J. Physiol. 372: 1986) of dye coupling between nerve-muscle contacts of cultured X. laevis cells prompted us to test Xenopus cultures for electrical coupling. The whole cell patch clamp was used with 400-G microelectrodes to resolve resolution high enough to record current through single gap junction channels. We used spontaneously occurring nerve-muscle contacts, or those which were created by manipulation of the muscle cell onto the nerve cell. Electrical coupling, measured by the flow of current from the nerve to the muscle was observed in a small fraction of nerve-muscle contacts. It remains possible that opened gap junctions, while not ubiquitous, may participate in inductive interactions associated with synaptogenesis. Supported by grants from the MDA, NSF, and NIH.

330.9
CELL SURFACE CONTACT INTERACTION DURING INITIAL STAGES OF SYNAPTOSIS. J. Chow, T. Hashab* and A.D. Grinnell. Jerry Lewis Neuromuscular Research Ctr. and Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

Direct cell-cell contact is important in synaptogenesis. There is evidence that this is enhanced by interaction between specific surface molecules, e.g. neuronal cell adhesion molecule (N-CAM) and developmental regulation which influences the timing or probability of synaptogenesis. This study was directed to assay the effect of inhibition of N-CAM homophilic cell-cell adhesion on the initial establishment of neuromuscular transmission.

When anti-N-CAM (kindly supplied by Dr. U. Rutishauser) was added to co-cultures of nerve-muscle pairs, whereas 60-70% of the pairs were MPP-positive in non-inhibited control cultures, 20% of the cultures were MPP-negative. Analysis of the effects of anti-N-CAM revealed that only 40% of the identified cholinergic neurons released ACh upon muscle contact. These preliminary results suggest that N-CAM binding may be involved in the initial cellular recognition and initiation of contact-induced neurotransmitter release in this system.

(Supported by grants from MDA and NIH)

330.10
IN SITU HYBRIDIZATION OF TUBULIN a-1 mRNA AS A MARKER OF NEURONS PARTICIPATING IN REACTIVE SYNAPTOSIS. J.W. Geddes, 1,2 C.W. Cormack* and F.D. Miller*. Dept. of Surgery and Psychobiology, Univ. of Calif., Irvine, CA 92717, and Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada.

Reactive synaptogenesis requires the extension of neuritic elements from neuronal populations which can be spatiotemporally restricted. Previously, we demonstrated that the mRNA for one isotype of a-tubulin, tubulin-ai (Tal), is expressed at high levels during the developmental extension of neurites. The level of expression is high in the adult brain, but can be induced during regeneration of facial motor neurons following a nerve crush paradigm. In contrast, the expression of a second a-tubulin isotype, Tα2δ, is unchanged during development and regeneration (Miller, F.D. et al., J.B.C. 105: 3065-3073, 1987). To determine if neurons undergoing increase their expression of Tal mRNA, we examined the intensity of the in situ hybridization signal to Tal mRNA at various times following lesion of the entorhinal cortex, utilizing 32P-radiolabeled anti-sense RNA probes transcribed from cDNAs cloned in pGEM4 vectors. In rats which received a unilateral entorhinal lesion, an increased hybridization intensity was apparent in ipsilateral entorhinal neurons adjacent to the lesion. The increase in hybridization intensity was apparent at both 1 day postlesion, a still apparent although significantly reduced by 14 days postlesion. These results demonstrate that similar molecular events may underlie neurite extension in development, regeneration, and synaptogenesis. The results further suggest that in situ hybridization of Tal mRNA may be a useful marker of synaptogenesis in human disorders including Alzheimer's disease, Down's syndrome and epilepsy. (Supported by the ADRDA, J.W. G. is a National Down Syndrome Society Scholar. F.D.M. is an AHPMR Scholar.).
SYNAPTOSIS II


Peanut agglutinin generally recognizes glycoconjugates(s) in the extracellular matrix at frog neuromuscular junctions (NMJs). To examine the role of these NMJ molecules (PNA-BM) synaptogenesis of rhodamine- or HRP-conjugated PNA was applied to muscles in tadpoles and bullfrogs (R. catesbiana). In early stages of development NMJ are distal from the basement. As development progresses, PNA background staining is reduced. Around metamorphosis, >90% of junctions showed colocalization of alpha- and PNA staining. As development progresses, PNA background staining is reduced. Around metamorphosis, >90% of junctions show colocalization of alpha- and PNA staining. Thus, NMJs, first in the clef, then also around Schwann cells are present. After Schwann cells appear, reaction products still are confined to the clef, but later appear also in the extracellular matrix around Schwann cells, as in adult junctions.

Thus, in synaptogenesis, PNA-BM initially are seen over the nerve and muscle surfaces but later are confined to NMJs, first in the clef, then also around Schwann cells. The appearance of PNA-BM in the initial nerve-muscle contact is not dependent on Schwann cells.


Three important changes that occur during neuromuscular junction development are an increase in acetylcholinesterase (AChE) levels, a decrease in the level of extrajunctional acetylcholine receptor (AChR), and an increase in AChR half-life (t½). Previously, we found that muscle activity regulates both AChR and AChE levels independently by decreasing intracellular Ca²⁺ (Ruben, L.L., PNAS 82:7121, 1985). Tetrodotoxin (TTX) inhibits contraction of cultured rat myotubes and prevents an increase in AChR levels; these effects are overcome by simultaneous treatment with Ca⁺⁺. Veratridine, which opens sodium channels but blocks contraction, promotes an increase in AChR, probably by stimulating a plasma membrane Na/Ca exchanger. Here, we report that muscle activity also appears to regulate AChR degradation rate. Treatment with TTX decreases AChR t½ relative to untreated cultures. Effects of TTX were overcome by the Ca⁺⁺-ionophores A23187 or ionomycin, which cause release of intracellular Ca and an influx of Ca through plasma membrane channels. Veratridine also increased t½ and decreased AChR levels. Curiously, cyanidine, which releases Ca from the SR, altered AChR and AChE levels, but did not affect AChR t½. This suggests that AChR and AChE synthesis can be modulated by Ca influx through plasma membrane channels as well as Ca release from the SR. AChR t½, however, appears to be regulated only by the former mechanism.


Synaptic plasticity induced by neuronal activation is thought to provide a physiological basis for learning and memory. Experimental models of activity dependent enhancement in neurotrophic-like experiments have relied on alterations in a number of synaptic parameters. Recently, activation of the NMMA receptor has been shown to be a critical step in the production of a plastic response by the synapse.

Over the course of development, the rapid increase in synaptic number is thought to correspond to the expansion of behavioral repertoire in response to the postnatal environment. Therefore, developmental synaptogenesis is also a model of learning and memory. The possible involvement of the NMMA receptor in developmental synaptogenesis was investigated using the selective NMMA antagonist DL-2-amino-6-phosphonovaleric acid (APV). Fifteen day old rat pups were intracranially administered APV via osmotic pumps. The pups were sacrificed two weeks after implantation (P30), cortical sections dissected out and processed for electron microscopy. Photographic analysis revealed a decrease in the number of synapses within the molecular layer of the neocortex in rats administered APV. This finding suggests that developmental synaptogenesis results from a mechanism similar to that producing synaptic plasticity in the adult.


Our laboratory has shown that chick muscle cells transformed by Rous sarcoma virus are unable to cluster acetylcholine receptors (AChRs) even after treatment with clustering factor (GF) derived from Torpedo electric tissue (Anthony et al., PNAS 84, 81, 2059-2063). Transformed cells are missing a 37 kd protein that is recognized by antibodies against muscle tropomyosins (Anthony et al., J. Cell Biol. 106:903, 1988). This protein is concentrated at neuromuscular junctions. To determine if this protein is involved in the formation and/or maintenance of AChR clusters, we microinjected a non-antibody FG (GIF)-label into transformed muscle cells. After injection, cells were treated with GF, incubated with rabbit antibodies to localize AChRs, then fixed and labeled with FITC-conjugated second antibody to identify injected cells. OS-16 injected cells had a decreased ability to form AChR clusters; the antibody did not disassemble the preexisting clusters. Cells injected with non-immune mouse immunoglobulin (IgG) were still able to form new clusters. To further test the role of the 37 kd protein, we disrupted clusters with sodium nitroprusside. After injection of GIF into washout, noninjected and nonimmune IgG-injected cells could form new clusters, but OS-16 injected cells could not. These data suggest that the 37 kd protein may play an important role in the formation of AChR clusters, probably as part of a sub-cluster cytoskeletal network.

TROPHIC AGENTS IV

331.1 EFFECTS OF BASIC FGF AND NGF ON SEPTAL NEURONS AFTER FIBRIFORM FIONIX TRANSACTION AND IN CULTURE - D. Otto*, M. Frotscher*, C. Gotthardt, and K. Unsicker. Deps. of Anatomy and Cell Biology, Univ. of Marburg, and Anatomy, Univ. of Frankfurt, F.R.G.

Basic FGF and NGF are present in the brain and trophic functions for the maintenance and transmitter metabolism of several CNS neuron populations have been assigned to them. We report here that both NGF and BGF are capable of reducing neuronal death in the rodent following unilateral fimbria fornix transaction (FTF) and enhance survival and choline transferase activity in cultured embryonic septal cells. NGF is mitogenic for a variety of factors or vehicle, respectively, to the FTF site. Cells counts performed on cresyl violet-stained sections after four weeks revealed no losses (0%) on the lesioned as compared to the unlesioned sides, which were significantly reduced by NGF (0.5 mg/75, 15 mg/54%) or BGF (5 mg/66%) treatments. Both factors sustained substantial proportions of Ch-Immunoreactive neurons, NGF and BGF also significantly increased ch activity in septal neurons. From 16 day old survival was only seen in low density cultures, but comprised both cholinergic and GABA-ergic neuronal populations. These data suggest a trophic role of BGF for sep- tal neurons in vivo and in vitro. Supported by grant # 34131 from the German Research Foundation.


The expression of nerve growth factor receptor (NGFR) mRNA was examined in the adult rat CNS through the use of in situ hybridization. Sense and antisense 332 labelled riboprobes were synthesized from a 320 bp EcoR1-BamHI fragment of the rat NGFR receptor gene inserted into a pT7/T7 transcription vector. Adult, Sprague Dawley rats (300-450 g) were used. The brains and superior cervical ganglia (SCG) were removed, blocked, and frozen in liquid nitrogen. Eight micromer frozen sections were cut and micrococaine-treated, gelatin-coated slides. The sections were fixed with 4% paraformaldehyde/PBS, rinsed, dehydrated and stored in a desiccator at room temperature overnight. Sections were hybridized with 10-50 ng sense or antisense probe (5 x 10⁹ cpm/µg) for 48-50°C. Sections were then treated with RNase-A (2.5-10 µg/ml), rinsed with SSC, dehydrated, dipped in Kodak NTB3 emulsion and stored at 4°C. After 10-30 days exposure, autoradiograms were developed and counterstained with cresyl violet.

Nerve growth factor (NGF) is a peptide that reportedly enhances survival of embryonic dorsal root ganglia (DRG) neurons and affects the duration of the action potential. In PC12 cells, it has been shown to induce the production of sodium channels.

In the present study, fetal human DRG neurons obtained from abortions of 16 to 19 weeks of gestation, were cultured in absence and presence of 40 nM NGF. After 1 week in culture, action potentials were recorded using the whole cell patch-clamp technique, in current clamp mode. Current steps of 0.1 nAmps with durations of 2 and 100 milliseconds were employed. At similar resting potential levels, cells grown in presence of NGF showed faster maximal rates of depolarization (+55.2%), of repolarization (+54.6%) and shorter duration (-20.6%) of the action potential compared to controls. The results indicate that NGF regulates the electrical activity of human fetal DRG neurons in culture.

331.4 FURTHER ANALYSIS OF NGF EFFECTS IN RATS WITH PARTIAL FEMORAL TRANSCTIONS. C.H. Montero, D.C. Mab, E.O. Huard, and F. Hefti (SPON: W.L. Strauss). Dept. of Neurology, University of Miami, Miami, FL 33101.

We have previously shown that intraventricular NGF administration to adult rats with partial femoral transections prevents the lesion-induced disappearance of septal cholinergic neurons. NGF treatment was not found to be equally effective in 18 month old female rats. Unilateral femoral transections reduced the number of ChAT and NGF receptor positive neurons in the septal area by 65% in old animals. In NGF treated old rats the decrease in cell number was only 11%. We also addressed the question whether the disappearance of cholinergic cell bodies represents morphological degeneration or simply down-regulation of cholinergic marker enzymes and NGF receptors. Septo-hippocampal neurons were retrogradely labeled with fluorescent dye before lesioning. Preliminary findings indicate that the lesion reduces the number of fluorogold labeled, ACh-positive neurons in the septum, suggesting cellular death after lesioning. Long-term but not short-term NGF treatment permanently rescued the cholinergic cell bodies from lesion-induced degeneration and stimulated regrowth of cholinergic fibers into the denervated hippocampus, suggesting that, after long-term treatment, the lesioned neurons had once again access to their source of endogenous NGF.
TROPHIC AGENTS IV
WEDNESDAY PM

331.9
ANALYSIS OF DELETION MUTATIONS IN THE NERVE GROWTH FACTOR RECEPTOR.
Nerve growth factor (NGF) is a polypeptide that exerts its action by interacting with
specific receptors on the surface of its target cells. Two populations of recep
tors exist on the surface of sensory and sympathetic neurons, as well as PC12 cells.
The two receptor populations are distinct in that one is insensitive to inhibition and
steady state binding of NGF. The major population of receptors releases NGF rapidly ("fast"
receptor) while the minor population releases NGF more slowly ("slow" receptor).
A cDNA clone for the rat "fast" NGF receptor was recently isolated. The protein
encoded by the cDNA clone contains a signal peptide and is rich in cysteine-rich elements,
two putative N-glycosylation sites, and a hydrophobic rich region which is probably a
membrane spanning region. In order to identify the amino acids needed by the
receptor for NGF binding, we made mutations in the cDNA clones, transferred the
mutations into mouse L cells, and determined the effect on NGF binding by the mutated
receptors. The first mutant is a deletion mutant containing the first 706 nucleotides of
the receptor clone, but lacking the portion encoding the hydrophobic rich region as
well as the carboxyl terminal end of the protein. The L cells expressing this receptor
had little NGF binding. These results suggest that the hydrophobic rich region is
a membrane spanning region and is needed to anchor the receptor in the mem-
brane. The membrane spanning region and the 3' end of the receptor might be di-
rectly involved in NGF binding or may influence the structure of the NGF binding
domain. In order to further delineate receptor sequences necessary for NGF binding,
we have constructed a set of deleted mutations that span several hundred nucleotides at
the 5' and the 3' end of the cDNA clone, commencing after the signal peptide, by exonuclease
III digestion. The deleted receptor clones were subcloned into an expression vector
for transfaction into mouse L cells. Characterization of these transfected cells in terms of
NGF binding will be discussed.

331.11
SOLUTION HYBRIDIZATION ASSAY TO MEASURE mRNA LEVELS FOR NERVE GROWTH FACTOR RECEPTOR.
and Pharmacology, University of Miami School of Medicine, Miami, FL
33101.
We have reported that treatment of septal cultures with nerve growth factor (NGF) increases the staining intensity of cholinergic neurons visualized with antibodies against the nerve growth factor receptor (NGFR). These results suggest that NGF stimulates the synthesis of its own receptor. In order to study the regulation of NGFR expression, we have developed a method to quantitate the amount of mRNA coding for this receptor using a solution hybridiza-
tion assay.
A cDNA clone for the rat NGFR (Raddeke et al. Nature 322: 593,
1987) was subcloned in pGEM-blue. A single stranded cRNA probe
(specific activity 8 x 10^6 cpm/mg) was transcribed using SP6 RNA
polymerase. This probe was hybridized (RoT = 0.008) to a sense
mRNA polymerase. After digestion with ribonuclease T1 and T2, the
nucleic acid resistant RNA was precipitated and the amount of specific
mRNA was quantitated. Based on this assay, the concentration of
mRNA coding for NGFR is less than 0.1 ng/mg of total RNA in the
basal forebrain of adult rats.

331.12
PRODUCTION OF A POLYCLONAL ANTISERUM RECOGNIZING THE NGF RECEPTOR.
A. A. Zapata*, P. A. Osborne*, and E. M. Johnson, Jr. Dep't of Neurology
and Pharmacology, Washington University, St. Louis, MO 63110.
Our laboratory recently described the presence of a truncated
form of the nerve growth factor receptor (NGFR) in the urine
plasma, and amniotic fluids of rats. We report here the presence
of forms of NGFR in human urine and amniotic fluid by utilizing
the human-specific anti-NGFR monoclonal antibody 20.4. 24k-NGFR
specifically bound to NGFR was chemically crosslinked using a
water-soluble carbodiimide. After immunoprecipitation, labelled
receptor species were visualized by autoradiography following SDS
PAGE. Labelled species corresponded to proteins of approximate
molecular weights 60, 50, and 37 kDa.
Employing human adult male urine as starting material, NGFR was
purified to near homogeneity by using a combination of ion
exchange and immunofinity chromatograms. Typical yields were
about one mg/L urine. The purified protein was NGF basal showed
specific binding to NGFR which was chemically crosslinked using a
water-soluble carbodiimide. After immunoprecipitation, labelled
receptor species were visualized by autoradiography following SDS
PAGE. Labelled species corresponded to proteins of approximate
molecular weights 60, 50, and 37 kDa.
Using the polyclonal antisera, we have shown that the antisera
reacts with the same protein bands in human urine and amniotic
fluid as we reported with the monoclonal antibody 20.4. The anti-
antisera also recognize denatured NGFR, various cell types were solubilized, run on SDS
polyacrylamide gels, and the proteins were visualized using the polyclonal antisera.
A single stained cRNA probe (specific activity 8 x 10^6 cpm/mg) was transcribed using SP6 RNA
polymerase. This probe was hybridized (RoT = 0.008) to a sense
mRNA polymerase. After digestion with ribonuclease T1 and T2, the
nucleic acid resistant RNA was precipitated and the amount of specific
mRNA was quantitated. Based on this assay, the concentration of
mRNA coding for NGFR is less than 0.1 ng/mg of total RNA in the
basal forebrain of adult rats.

331.13
IN VIVO EFFECTS OF GM1 AND NGF, ADMINISTERED IN COMBINA-
TION, FOLLOWING CNS RETROGRAD DEGENERATION.
C. Garofalo, B. Neuringer and A.C. Cuillo, Dept. Pharmacol-
ogy, McGill University, Montreal, Canada, H3G-1Y6.
Following a unilateral decortication, retrograde degeneration changes occur in the nucleus basalis mag-
noceullaris (NBM) of mature rats which can be prevented by administering the monosialoganglioside GM1. Nerve
growth factor (NGF) administered via minipumps (12
ug/day, 7 days) is equally effective. We have presently been examining the possibility that GM1 may exert its ef-
fects by preventing the retrograde effect produced by chronically administered NGF (60
ug/day, 7 days). The effects of NGF on the morphology of the neurons was studied using autoradiography following
silver staining of the section. The results show that although NGF prevents the degeneration of the NBM,
NGF prevents the retrograde transport of NGF from the periphery to the brainstem. These results suggest that
NGF may be a neurotrophic factor for the neurons of the NBM.

331.14
RETOGRADE TRANSPORT OF NERVE GROWTH FACTOR BY MOTOR
NEURONS OF DEVELOPING RATS: ASSESSMENT OF POTENTIAL
NUEROEFFETIC EFFECTS. W. Snider, Q. Yan, J. Piocone and E. M.
Johnson, Jr. Dep't of Neurology and Pharmacology, Washington Univ.
Sch. of Med., St. Louis, MO 63110.
Several regions of the central and peripheral nervous system transiently express receptors for nerve growth factor (NGF) as revealed by a specific monoclonal antibody to the rat NGF receptor (Yan and Johnson, J. Neurosci., in press). In the spinal cord, staining is most intensely associated with central motor neurons that are observed from about E15, peaking on the day of birth, and decreasing to undetectable levels by postnatal day 10. In order to assess the function of these receptors, we have studied the retrograde transport of NGF by spinal motor neurons and the responses of motor neurons to pharmacological doses of NGF in neonatal rats.
125I-NGF was retrogradely transported by motor neurons from their peripheral nerve terminals in newborn animals. This transport was blocked by an excess of unlabeled NGF but not by cysconehcine. 125I-Cysconehcine was not transported. The monoclonal anti-rat NGF receptor antibody was also transported, but not a control antibody. Despite this ability of motor neurons to transport NGF, treatment of neonatal rats with this factor did not increase motor neuron size or synthesis of neurotransmitter enzymes and did not prevent cell death after axotomy. We conclude that NGF receptors on motor neurons can bind, internalize, and retrogradely transport NGF. However, these receptors do not mediate the morphological and survival-promoting effects associated with the action of NGF on sympathetic and dorsi
root ganglion cells.
331.15

The expression of nerve growth factor (NGF) receptor was studied in embryonic rat skeletal muscle by electron microscopic (EM) and light microscopic (LM) immunocytochemistry using 192-IgG, a monoclonal antibody specific for rat NFG receptor. NFG receptor immunoreactivity (NGFRI) in skeletal muscle was seen as early as embryonic day 11 (E11) but was most intense in E16 animals. In E15 rats the NGFRI seen by EM was localized to the cell surfaces of two cell types. Myotubes with and without defined myofibrillar cytoplasmic components had intense NGFRI. Cells that were less obviously differentiated, with a spindled, fibroblastic appearance, also were heavily immunostained. By EM the NGFRI in E18 rats was absent or only faintly present on the more well-differentiated muscle cells. At E18 there were numerous spindle-shaped cells with intense NGFRI interspersed among the fibri-containing muscle cells. By the time of birth the NGFRI seen by EM was greatly diminished and by EM appeared to be confined to the spindle-cell population. The transient expression of NGF receptors by skeletal muscle during development appears to be correlated with the cellular differentiation. This might play a role in cellular mechanisms related to the fusion of myoblasts into myotubes and subsequent myofibrillar differentiation into mature myocytic sarcomere.

331.17

Nerve growth factor (NGF) has recently been implicated as a trophic agent in the maintenance and maintenance of the basal forebrain cholinergic neurons. Evidence from animal studies suggests that a continuous intraventricular infusion of NGF or the intracerebral grafting of NGF producing cells into the Alzheimers’ brain may slow the degeneration of the neurons and improve memory function. For this purpose human NFG (hNGF) expression vectors were constructed to allow for the production of recombinant hNGF for infusion and also for the ability to transform cells for NGF delivery. Three expression vectors were constructed containing the entire coding region for mature (13,000 Da) NGF. Vector 1, a pBluescript vector containing the metallothionein promoter, consists of a 1200 bp Hind III-Bcl I fragment joined to a linking oligo eluding the 30 bases of the 5’ end. Vector 2, a pXam vector derived containing the AMLP promoter, consists of a 1050 bp Ava’l-Apal hNGF fragment. Vector 3, a pGEM-3 vector containing the staphylococcal coagulase gene, consists of a 1200 bp Hind III-Bcl I 1 NGF fragment joined to a 255 bp fragment of mouse NGF cDNA. All three NGF cDNA fragments contain the two potential initiation codons at -122/129 and with the mouse NGF cDNA fragment of vector 3 also supplying the 5’ sequences up to the potential initiation codon at -187. All vectors were selected and shown to contain the respective inserts by restriction mapping and oligonucleotide hybridization. The vectors are being transiently expressed in COS, CHO and PC12 cells. Expression will be measured by analysis of the transfected cell medium for immunoreactive and bioactive NGF. It is hoped that the expressed NGF may represent an important first step in the potential treatment of Alzheimer’s disease.

331.18

The nucleotide sequence of a rat β-nerve growth factor (NGF) genomic sequence encoding the entire 417 amino acid preproNGF was determined. Rat NGF shows very high homology with other known NGFs in both 3’ untranslated regions and the prepro-peptide regions. The mature NGF, which is glycosylated, contains cysteine residues important for tertiary structure, glycrosylation sites and dibasic amino acids required for proteolytic cleavage of the preprotein. NGF and hNGF are homologous. Comparison of hydrophobicity plots and amino acid sequences revealed an evolutionarily divergent domain on the external surface of NGF which may account for the poor immunologic cross-reactivities of the various NGFs. In situ hybridization to brain sections with a rat-specific oligonucleotide indicated high levels of NGF mRNA synthesis in both hippocampal granule and pyramidal cell layers. These results are consistent with one role for NGF in the CNS as a neurally-released, retrogradely transported neurotrophic factor for basal forebrain cholinergic neurons. Labelled cells were also observed in olfactory cortex and other CNS regions are presently being examined.

GENETIC MODELS II

332.1
SEVERITY OF CORPUS CALLOSUM DEFICITS IN TWO SUBSTRAINS OF BALB/c mice in relation to uterine location of fetuses. B. Boulton-Fleming and D. Wahlsten. Dept. of Psychology, U. of Waterloo, Waterloo, Ontario, Canada NZL 3GI.

Intracerebral position (IP) effects were studied using fetuses at embryonic day 17.5 from two substrains of BALB/c mice in order to determine the effects of IP on the development of the corpus callosum. The results revealed: 1) differences between the lines in mean litter size, body weight, and score; 2) a right hand effect, with high scores in line 1; 3) an ovarian and cervical (vs. middle) position advantage in line 2 for body and placenta weight. Apart from the line 2 right hand effect, no other IP factor or combination of factors contributed significantly to the variability in degree of CcCH retardation. A runs test for random- ness also provided no evidence for nonrandom placement of fetuses with respect to degree of abnormality.

332.2

In the Sprague-Dawley rat colony of our Department we found a neurological mutation in a group of trimed rats characterized by tremor, ataxia, catalepsy and paralysis. The syndrome begins with mild tremor, then progresses to more severe tremor and hindlimb paralysis, visible by simple inspection when male rats are 1 month old. Other neurological symptoms appear with increasing age: ataxia at 2-3 months and hindlimb paralysis at 10 months. This neurological syndrome is transmissible as an autosomal recessive trait.

The tremor frequency was measured in 8 or more mutant rats at the following ages: 19, 21, 27, 30, 45, 60, 75 and 90 days, using power spectral analysis of the current induced by the movements of a magnet, attached to the animal, on a wire coil wrapped around the rat according to the method described by Shimozaki (Neurosci. Res. 2:92-100, 1984). A peak frequency of tremor was detected with a mean frequency of 13.4 ± 2.8 Hz. At the time of 21 days all mutants exhibited tremor at an average frequency of 13.4 ± 2.8 Hz. In 45-60 day-old rats, a second tremor peak appeared at 6.4 ± 0.2 Hz, which then increased, reaching 16 Hz at 90 days. This second peak above 10 Hz was significant in the tremor of the age and sex. This study was supported by a grant from the National Science Foundation.
332.4

Avian muscular dystrophy (MD) in the Davis line 413 dystrophic chickens (DC) has been used for the study of hereditary MD in human muscle. Muscle Nerve, 10:168, 1987. We investigated these variants in 5-week-old and 6-month-old male DC. Muscle (Ca) and (Mg) were determined in the MD by extract by flame atomic absorption techniques (Anal. Lett. 12:1451, 1979). Age and sex matched Davis line 412 normal chickens (NC) served as disease controls. Unlike DMD and DH, EICA and gross morphological changes with profound cellular necrosis and fatty infiltration were not evident in the myocardium of DC. However, the pectorals from DC revealed significant EICA accompanied by my depletion and classical dystrophic histopathology. Plasma CK activity was also elevated in DC (p < 0.001).

We conclude that DC exhibit lesions in the skeletal muscle universe permanently supported by NIH grant AR-38540.

332.5
IMMUNOHISTOCHEMICAL LOCALIZATION OF HPRT IN THE MAMMALIAN BRAIN. M. H. Brilliant and M. Lennon-Pierce*. The Jackson Laboratory, Bar Harbor, ME 04609.

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) is an enzyme responsible for converting the bases hypoxanthine and guanine into their respective nucleotides. Deficiency of HPRT in humans results in Lesch-Nyhan syndrome, characterized by several neurological signs including excessive spasticity, choreoathetosis and self-injurious behavior. In mice, however, absence of HPRT causes no obvious neurological impairments. In view of this difference, it would be interesting to compare the distribution of HPRT in the brains of normal and aneuploid mice. HPRT was localized in sections from murine brain using rabbit anti-human HPRT, and visualized by standard immunoperoxidase techniques.

The regional distribution of labeled cells was quite specific; all cells were not uniformly stained. The most intensely stained cells were located in the olfactory tubercle/fetal cortex, anterior colliculus, and deep layers of the superior colliculus. Medium intensity staining was observed in specific cortical layers, amygdala, several hypothalamic nuclei, red nucleus, all brainstem motor nuclei, deep cerebellar nuclei, and nuclei of the trapezoid body. The cellular distribution of label varied according to brain region. In some areas, whole neurons were densely stained; with clearly identifiable dendrites. In other regions, staining appeared coarsely granular, not evenly dispersed within the cell cytosol. Granules often appeared clustered at the cell surface, but it remains unclear if they represent intracellular inclusions or exteriorly located synaptic puncta.

We are currently investigating the distribution of HPRT in the brains of human and non-human primates.

332.6
GENETIC MAPPING AND DEVELOPMENTAL EXPRESSION OF GENES ON MOUSE CHROMOSOME 19 IN NORMAL AND ANEUPLOID MICE. R. F. O'Hara*, R. H. Reeves*, C. Bendotti*, S. Fisher*, C. Toye, M. L. Oster-Granite, and J. D. Gearhart*, NCI, National Cancer Institute, Bethesda, MD 20892, and the Department of Pathology, University of California, Davis, CA 95616.

The cellular distribution of label varied according to brain region. In some areas, whole neurons were densely stained; with clearly identifiable dendrites. In other regions, staining appeared coarsely granular, not evenly dispersed within the cell cytosol. Granules often appeared clustered at the cell surface, but it remains unclear if they represent intracellular inclusions or exteriorly located synaptic puncta.

We are currently investigating the distribution of HPRT in the brains of human and non-human primates.

332.7

GAP-43 is a neuronal protein associated with axonal elongation during development, with regeneration following injury, and with synaptic modification and plasticity in the adult central and peripheral nervous systems. The expression of GAP-43 increases during fetal and early postnatal life, and then decline, so that adult levels are considerably lower than those found during development in normal animals. O'Hara et al. (this meeting) have localized the gene encoding GAP-43 to a region of mouse chromosome 16 (MMU 16) homologous to human chromosome 3.

We have used in situ hybridization and Northern blot analysis to determine the developmental expression of GAP-43 mRNA in normal and trisomic mice. In situ hybridization was done at E10 in normal brains (15) and at E15 in trisomic brains (16) to study the effects of the increased gene dosage on the expression of GAP-43 mRNA. Mice trisomic for MMU 19 (Ts19) served as controls for the generalized effects of aneuploidy on development. As early as day 11.5 of gestation (E11.5) in normal mice, GAP-43 mRNA was detected in whole head preparations; expression increased in the brain approximately 10-fold by postnatal day 1 (PND 1), and continued to increase during the first postnatal week. GAP-43 mRNA can be detected as early as E10 in normal mouse brains by in situ hybridization. At E13, GAP-43 mRNA expression was increased 2-3 fold in Ts16 mouse brain, relative to euploid littermate controls, but was decreased slightly (20%) in Ts19 mouse brain, relative to their euploid littermate controls. Alteration in the expression of GAP-43 mRNA observed in these aneuploid mice may contribute to some of their CNS developmental abnormalities.

332.8
DEVELOPMENTAL EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN NORMAL AND TRISOMIC 16 MICE. RELEVANCE TO DOWN SYNDROME AND ALZHEIMER'S DISEASE. S. Fisher*, R. A. Morgan*, B.F. O'Hara*, M.L. Oster-Granite, and J. D. Gearhart*, Developmental Genetics Lab, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The A4 peptide is a major component of the cerebrovascular amyloid plaques and extraneuronal senile plaques found in the brains of Alzheimer's disease patients and aged Down's Syndrome mice. The gene encoding the amyloid precursor protein (APP) maps to human chromosome 21, and its homolog is mouse chromosome 16. We have cloned a full-length mouse APP cDNA, which has a 97% homology with the human form at the nucleotide sequence. The human APP gene is composed of 5 exons and 4 introns. The expression of APP in human and mouse brains during embryonic development has been studied by RFLP analysis and by examining the expression of MMU 16 specific genes during development in normal and aneuploid mice. The detailed pattern of APP and G A P -4 3 m R N A  expression on postnatal day 1  (P N D  1), and continued to increase during the first postnatal week. G A P -4 3 m R N A  can be detected as early as E15, G A P -4 3 m R N A  observed in these aneuploid m ice m ay contribute to some of their CNS developmental abnormalities.

d is o rd e r, de rived from the o r ig in a l W ista r breeding sto c k , have
on LM there was an apparent in cre ase  in
cords of these o ld e r md r a ts , compared to mutants o f 30 days o f age.
J .  V .  C l i f t o n * . D e p t . o f  P s y c h o l o g y ,  U n i v .  o f
m o tio n  r e c o r d  w ere a n a ly z e d  u s in g  t r a d i t i o n a l  s p e c t r a l


T a i l  l e n g t h  a t  w e a n i n g  ( 1 0 -9 0 %  o f  n o r m a l )  i s
a f f e c t i n g  t a i l  a n d  C C  m o r p h o l o g y  h a s  b e e n  d e t e c t ­
a nalysis showed that the PLP mRNA, a single band of 3.2 kb, is
detectable in the brain and spinal cord of both normal dogs and
shaking pups by two weeks of age. The PLP mRNA is the same
size in the shaking pup as in the normal dog, but is reduced to
about 10% of normal levels. Southern blot analysis indicates that
there are no gross deletions or rearrangements of the PLP locus
in the shaking pup. Genomic DNA libraries from normal dog
and shaking pup were constructed in the EMBL3 vector and screened
with a human PLP cDNA. The PLP clones were mapped and
sequence analysis is underway to locate the defect in the shaking
pup PLP locus. (Supported by grants from the NIH (NS23124) and
NMSS (RG1791) to IDD).

FREQUENCY ANALYSIS OF WHOLE BODY OSCILLATION IN SHIVERER (SH) MUTANTS. D. Pancaro, P. Tai, M. C. Sandberg, E. D. Molenaar, and P.A. Keeney, Division of Neurology, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

Glycophospholipids play major roles in many functions like cell migration and adhesion, which are involved in neurulation. Previous studies by our laboratory have indicated correlations in the time of appearance, type and distribution of glycosphingolipids with normal as well as with teratogenic-induced (vitamin A) neurulation. We suggest that glycosphingolipids may serve as molecular participants in the processes of neurulation. In the present study, a genetic model of abnormonal neural tube closure, the delayed-shaking mouse mutant (sh/kc) was studied on gestation days 11, 14, and 17 by battery of FITC-labeled lectins using light-low intensity video-microscopy and by analysis of micro-dissected neuruplasms (NB) using PAGE, western blots, and peroxidase-coupled lectins to detect carbohydrate composition. The gestation day 16 Sp/SP embryote exhibited varying degrees of myeloschisis of the sacral region, and concomitant changes were observed in the staining patterns of several FITC-labeled lec­
tine. Image analysis of FITC-WGA binding by control NE in 1-mm plastic sections exhibited spatial and temporal changes during neurulation, particularly in the zones of closure; FITC-WGA binding was allowed in the NE of Sp/Sp embryo. Western blots demonstrated a decrease in the WGA-binding of 16-Kd and 80-Kd proteins in both the caudal and neural NE in gestation day 16 embryos with myeloschisis compared to intermesencephalon. The protein profiles of Sp/Sp, intermesencephalon, and control embryos were similar on silver-stained gels, i.e., no changes were observed in the 16-Kd and 80-Kd proteins. The results of this study demonstrated change in glycosphingolipids in Sp/Sp embryos, these changes were similar to the abnormalities of the shaking mutant and vitamin A-induced myeloschisis in the mouse. Thus, these results indicate that glycosphingolipids may be molecular partici­pates in the events of abnormal neurulation and provide additional evidence for the impor­tance of glycophospholipids and the process of neurulation.
332.15

The aim of this study was to determine whether anomalies of frontal lobe organization and frontal cortex system in Down syndrome (DS) occur during the infancy and early childhood. We applied CHAT-immunochemistry (anti-human placental CHAT antibody of Hersh-Bruce), AChE-histochemistry and Nissl method, analyzing basal forebrain (BF) and frontal cortex on postmortem tissue obtained from 5 individuals with DS and age-matched controls (newborns–7 years).

In newborns, more than 80% of BF neurons are CHAT-reactive in both DS and controls; CHAT-reactivity was enhanced in DS basal forebrain. Cortical cytoarchitectonics and AChE-patterns appear to be normal. In older group (2–5 years) ACh-reactive pyramidal neurons were not found but fibre staining was enhanced. The pyramidal neurons showed a vacuolar degeneration. In both age groups the mean perikaryal size was not different in comparison with age-matched controls. In conclusion, basal forebrain-cortical system appears structurally normal in the newborn, with possibly increased CHAT-synthesis. At 2.5 years first abnormalities appear in cortical associative layers, indicating that cortical pathology may be a primary phenomenon.

332.16

Elevated levels of norepinephrine (NE), mainly in cortical regions of the brain, have been reported to be responsible for petit mal and focal motor activity in tg/tg mice. However, thorough analysis of NE levels in the brainstem and spinal cord have not been previously done. Since these CNS regions may also be important in the generation and/or maintenance of these forms of seizures, NE levels were measured via HPLC-EC in several brainstem and spinal cord regions in the adult tg/tg mice relative to control (+/+) mice. Decreased NE was observed in the pons from tg/tg mice while other brainstem and spinal cord regions exhibited increased NE levels.

Methionine-enkephalin (MET-ENK), known to colocalize with and modulate the actions of NE, was also measured via RIAs in the brainstem. In those areas NE was decreased intg/tg mice. MET-ENK also was decreased, relative to +/+ mice. These data raise the possibility that decreased NE and MET-ENK may be involved in epileptogenesis and/or maintenance of seizures activity in the tottering mouse.

Measures of MET-ENK levels in spinal cord regions are in progress. Supported by NIH grants R01NS15 and R05-66255.

333.1
PATTERNS OF RETINAL TERMINATIONS IN EYED SUPERIOR COLLICULUS LABELLED WITH HRP AND WGA-HRP TRACERS. S. Agarwala, H. W. V. Nicholas, R. M. Petry.

Dept. of Psychology, S.U.N.Y. at Stony Brook, NY 11794

Retinal projections were examined after monocular intravitreal injections of either horseradish peroxidase (HRP, 50 ul of a 30% solution) or wheat germ agglutinin conjugated HRP (WGA-HRP, 50 ul of 1% solution) in ground squirrel, sci-174. Following various survival schedules, brains were processed for TMB histochemistry.

The retinal projection patterns resulted from injection of either HRP or WGA-HRP were similar for all structures except for the contralateral superior colliculus (SC). In ground squirrel, this projection exhibited a discontinuous pattern of vertical slabs throughout the superficial layers in all HRP cases and in some WGA-HRP cases. However, uniform labeling of the contralateral SC occurred in other WGA-HRP cases. These traces also produced similar differences in the pattern of labeling in the rat SC; the differences could not be attributed to obvious methodological factors, nor could they be adequately explained by superior WGA-HRP sensitivity or its transneuronal transport. The similarity of labeling in all visual projections regardless of tracer, makes the differences in the SC projection very intriguing. (Supported by NSF grant BNS 8519023 to JGM and NIH grant R29-EY07113 to MMP).

333.2
EFFECT OF LESIONS INVOLVING THE PARABIGEMINAL NUCLEI ON CHOLINE ACETYLTRANSFERASE AND ACETYLCHELINESTERASE ACTIVITIES IN RAT SUPERIOR COLLICULUS. C.D. Ross*, W.B. Farms*1.

Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Activities of choline acetyltransferase (CAT) catalyzing the synthesis of acetylcholine (ACH), and acetylcholinesterase (AChE), catalyzing the destruction of ACH, are high in the superior colliculus (SC). In collagenase-dissected superficial layers that receive substantial projections from the parabigeminal (PB) nuclei. Activities of CAT and AChE were assayed in dissected microstrips from the superficial layers of the SC and in cerebellar collayers in rats killed one week after electrolytic lesions were made to areas including the PB. Lesions destroying PB bilaterally decreased PB activity in ChAT activity in superficial SC layers, with a slight (about 20%) decrease in some deeper layers, but no significant change in ACH activity. Lesions of similar size, but rostral to and sparing the PB, produced no change in activity of either enzyme. These results indicate that a substantial cholinergic projection to the superficial SC layers originates in the PB. ChAT activity in the SC is not associated with the optic projection, since it does not decrease following enucleation. Therefore, ChAT activity remaining after PB destruction could be related to a small projection from another source or to intrinsic cholinergic neurons in the SC. (Supported by NIH grant EY-03830)

333.3

Horizontal (H) cells are a prominent cell type in the superficial layers of the rodent SC. The orientation of the dendritic arbors of these neurons has been the subject of some controversy. Based on our examination of Golgi material, Valverde (G. Anat. Embryol. Gech., 147:117, 1973) concluded that the dendritic arbors of H cells in the hamster were perpendicular to the surface of the retina which is also the case in the rat. Langer and Lund (J. Comp. Neurol., 158:605, 1974) suggested that the orientation of H cells dendrites paralleled isodisocyan and low eccentricities of the representation area in the rat’s SC. Tokunaga and Otani (Exp. Neurol., 52:189, 1976) concluded that H cells in rat have no preferred orientation. We have also reconstructed horizontal connections peroxidase-filled neurons to determine whether H cells in hamster have a preferred dendritic orientation. In 9 of 10 horizontal cells, the mediolateral extent of the dendritic tree exceeded its rostrocaudal dimension. The average ratio of these two dimensions was 2.1 (±1.2). The long axis of H cell dendritic arbors was oriented at an angle of 20.1° (±13.2) to the frontal plane. Thus in hamster, H cells do not have a preferred dendritic orientation and is generally parallel to the frontal plane. Supported by BNS 85-00142, EY 04170 and NS 07229.

333.4
INTERLAMINAR PROJECTIONS IN THE HAMSTER’S SUPERIOR COLLICULUS. R.G. Mooney, S.E. Fish, M.M. Nikolatseas, W.H. Rohrer and E.W. Rhodes. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Marshall University Medical School, Huntington, WV 25700

We used Phaseolus vulgaris-leuco-agglutinin to trace interlaminar projections within the superior colliculus (SC) of normal hamsters. The superficial SC laminae projected to all of the deeper layers and to the periarcuate gyrus. This projection terminated most heavily in the stratum griseum intermediate and stratum griseum profundum and was sparse in the stratum griseum progundum. There was also a projection from the deep to the superficial laminae, but it was relatively sparse. The trajectory of the superficial laminae projection could be followed along two courses: a medial, angled laterally from a line that extended orthogonally to the SC surface from the injection site center. We assumed each orthogonal to be the most likely trajectory of sections projection lines, i.e. lines connecting the centers of receptive fields that have constant elevation and azimuth in the visual field. In some of this apparent mismatch, we combined PRA-L tracing with receptive field mapping to determine how well the trajectory of the SC-interlaminar pathway matched actual projection lines the superficialic and deep layers. As the case for the trajectory of the SC-interlaminar projection, the projection lines in the hamster’s SC were angled laterally from lines orthogonal to the SC surface. Supported by BNS 85 00142, EY 04170 and NS 07229.
333.5 PRESYNAPTIC TRANSFER PROPERTIES OF THE RETINOTECTAL CONDUCTION SYSTEM IN THE SUPERIOR COLLICULUS: BRACHYUM OF THE RODENT: D. L. Lippeman and D. Fox. Dept. of Physiology, UT Southwestern Medical School, Dallas, TX.

Previous electrophysiological studies have shown that the fast conducting t1-Y/like (12,5nA/s) and the middle conducting t2-X/like (6,0nA/s) axons branch to innervate both the superior colliculus and the dLGN(Vis.Basal:8,867). HRP retroinnexial studies have demonstrated that the major axonal projection to SC(6.3%) is comprised of the slow conducting (3.5nA/s) t3-U/like axons. We have studied the presynaptic transfer properties of retinotectal axons and have studied their functional characteristics in OT and SC brachium depth recordings. The data show that 1) the relative amplitude measurements of t1, t2 and t3 are better correlated with SC HRP estimates of the BCC conduction group projections than with unit latency histograms; 2) orthodromic t1 conduction is preferentially slowed (15-20%) in brachium recordings; 6t2 and t3 brachium responses are different functions are depressed to the same extent which parallels the SC postsynaptic field potential response. The t3 response depression is sensitive to the SAP channel blocker suggesting that accumulation may contribute to the SC field potential postsynaptic depression. Supported by NIH NS Grant 30183(DAF).

333.7 AMPHETAMINE INCREASES RECEPTIVE FIELD SIZE IN THE SUPERFICIAL LAYERS OF CAT COLLICULUS: K. Grams1, R.M. Lee2, and J.R. Mendlson. MD. Dept. of Psychology, York University, North York, Ontario, Canada and 3Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, B.C., and 3Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada.

Visual responses were examined in the superficial layers of the superior colliculus (SC) of anesthetized, paralyzed cats before and after (i) administration of dextroamphetamine sulphate (40mg/kg i.p. through the external carotid artery only) for a number of visual response properties receptive field (RF) size and position, direction selectivity, strength of inhibitory surround, and the incidence of 'on' and 'off' responses: (ii) 10 minutes to 1 hour after amphetamine injections (2ml of 10mg/ml). RF size began to gradually increase. RPs expanded by some 2-10 times (mean 4.5) over the next 2-4 days. SAR decreased (i.e., 4-7 hours post-injection). RF size returned to pre-injection dimensions. In most cases, RF size did not increase equally in all directions, but rather displayed asymmetrical patterns of expansion. In addition to changes in RF size: 1) responses to flashed stimuli became much more vigorous; 2) surround inhibition, when present, usually because much weaker; and 3) no consistent effects were noted in direction selectivity. It is possible that the elevated activity levels in the SC resulting from increases in RF size, will affect the peak velocity and/or timing of visually evoked saccades.

333.9 MULTIPLE VISUAL MAPS IN DEEP SUPERIOR COLLICULUS OF CATS: M.A. Meredith and B. E. Stein. Dept. of Anat. and Physiology, Medical College of Virginia/VCU, Richmond, VA 23298.

Topographic register among sensory representations is thought to be a critical factor in facilitating coordinated responses to a variety of sensory cues. Such sort of multisensory register has been demonstrated in the superior colliculus (SC) by relating the superficial layer retinal maps of the deep laminae to the body and auditory space. Yet, because of the differing afferent and efferent connections of these laminae and their differing behavioral roles, this superficial-deep layer sensory register may be of little functional significance. In the present experiments, the deep laminae visual representation. Unimodal visual neurons (n=52) had receptive fields (RFs) of intermediate size with nasal-temporal borders varying with AP and elevation within the SC. In contrast, visual neurons that received auditory inputs (n=76) had significantly larger RFs whose nasal-temporal borders varied with AP but whose elevation was generally insensitive to different ML locations. These data demonstrate the sharing of axes by visual and nonvisual representations in the deep laminae. In addition, the differing patterns of visual RF distribution among unimodal and multisensory neurons, coupled with their different efferent projections, suggest that superficially and functionally distinct visual maps coexist in the deep laminae. Supported by NS-22543.


91 intracellular recordings were made from tectal neurons of 26 adult goldfish using HRP filled microelectrodes. Resting potentials varied between 30-70mV; action potentials ranged from 100-140mV. A 5 sec flash provided visual stimulus. Following iontophoretic injection of HRP, 36 sites were identified in histology sections. 21 identified unit injections corresponded to the following morphological types described by Meek and Schellart (1978); 4 large diameter afferent fibres; 6 type I cells (pyramids); 1 type III cell; 1 type VI cell; 7 cells with somas in the DPL - type IV and 1 type XV cell and 2 glia cells. Afferent fibres provided 'on' responses, either phasic, tonic or bursting without spontaneous background; type 1 cells mostly yielded 'on' dominated responses and with weak or negligible 'off' components. The spontaneous activity consisted of regular bursts. One cell did not respond to light. Type III cells provided delayed 'on' response, while the type VI cell produced an irregular response suggesting equally strong 'on' and 'off' components. Responses from SFV cells were variable. Glial cells appeared silent, but type XIV and XV cells were either silent or spontaneously active at a low or high level. Responses were phasic or phasic (delayed) 'off' or tonic 'on'-off'. Supported by NEI 01426.

333.8 CONTRIBUTIONS OF THE SUPERIOR COLLICULUS OF THE MONKEY TO VISUAL SPATIAL ATTENTION: Caroline Kastmans and David Lee Robinson Laboratory of Sensorimotor Research, National Eye Institute, NIH, 31 Center Drive, Bldg. 10, WO 8825, Bethesda, MD 20892.

Visual spatial attention refers to the ability to select images for special use independent of eye movements. We have trained rhesus monkeys on a task developed by Posner: the animal fixates a spot of light and contacts a bar when a target light appears. Reaction times are faster for validly cued targets than for invalidly cued ones. When we injected muscimol into the superior colliculus, the animal was slow in responding to validly cued targets in the visual field contralateral to the injection, suggesting a spatially delayed stimulus. Tone stimuli were spatially delayed for the same target when it was preceded by a cue in the other hemisphere. The authors argued that it had difficulty with visual spatial attention. In control monkeys, neurons in the superficial layers of the superior colliculus were easily driven in this task. Cells in the foveal representation responded when the fixation target was in the neuron's receptive field: there was no change in activity associated with the cue (which is hypothesized to shift attention). When the cue and/or target were positioned in the visual receptive field of a neuron, cells were driven briskly. Disregarding simple sensory factors, there were no consistent and significant changes in the response of a cell to a target dependent on cue validity. The effects of pharmacological manipulation of the superficial colliculus, however, show that this structure contributes substantially to visual spatial attention. These data show that the tectum has a visual function which is independent of eye movements.

333.10 SPATIAL CHARACTERISTICS OF MULTISENSORY INTEGRATION IN BEHAVING CATS: Lawrence McDaniel, M. Alex Meredith and Barry E. Stein (SPOK: Karl C. Corley). Deps. of Physiology and Anatomy, Medical College of Virginia/VCU, Richmond, Virginia 23298.

Our previous studies have shown that a simple spatial rule characterizes multisensory integration at the cellular (superior colliculus) and behavioral levels: coincident multisensory cues produce enhancement whereas disparate stimuli produce depression (or no effect). In the present studies, we evaluated the resolution of this system behaviorally by determining the spatial separation between stimuli necessary to convert enhancement to depression. Three cats were trained, oriented and acquired a low intensity visual stimulus at various positions along a semicircular track. A low intensity coincident auditory stimulus was presented at the same stimulus inhibited correct responses when located at little as 15 degrees medial to the visual. When the auditory cues were presented lateral to the visual, it not only failed to depress correct responses but often enhanced them. This medially lateral difference may be due to the large excitatory receptive fields in multisensory neurons which allow stimuli to be presented to one side of the auditory receptive fields in multisensory neurons which allow still produce excitation in the same auditory-receptive neurons. Supported by NIH Grant NS 22543.
The infrared sensitive pit organ of creodont snakes sends spatial information to the optic tectum via a direct projection of the suprachiasmatic nucleus. We are interested in identifying tectal cells that integrate visual information and infrared (IR) information from the pit as a step towards describing the infrastructure of multisensory integration.

We have recorded intracellularly from visual and IR terminals and postysnaptic cells in the tectum of oldfield mice (Smith et al, 1986). The infrared afferents (from corneal receptor fibers in the brachial stem) are large diameter fibers arborizing extensively (<500 μm in ant/post and 500-1000 μm med/lat) in the stratum griseum superficiale (SGS); smaller fibers were found at this site. The fiber size does not seem to be reflected by IR receptive field sizes of postysnaptic cells. Terminal arbors of the large fiber fibers are smaller and are found above SGS in stratum fibrosum et griseum superficiale.

OK neurones, which responded to both visual and IR inputs had dendrites that arborized in both superficial and deeper tectal layers. Unstimulated visual or IR cells were rarely encountered, probably because of their small size.

**THERMAL DEPENDENCE OF NEURAL ACTIVITY IN THE HAMSTER**

High temperature just below which a population spike could be recorded, T

1 was 4 degrees lower in a hibernator, the gopher, than in a nonhibernator, the rat (Hooper et al., J. therm. Biol. 10:35, 1983). In addition to this study showing phylogenetic adaptation, in a study on acclimation to cold (Thomas et al. J. therm. Biol. 11:23, 1986) showed that T

1 for hibernating hamsters was 15:3 ± 0.5°C compared to 15:8 ± 0.5°C for non-acculturated hamsters. In this study the amplitudes of population spikes evoked by Schaffer collateral stimulation (using methods described in papers cited) were measured in non-acculturated hamsters and rats as bath temperature was lowered from 35°C to T

1. As temperature decreased the amplitude increased to a maximum and then decreased in both hamsters and rats. Long term potentiation (LTP) was evoked above 22°C by pulse train stimulation (10 trains at a rate of 3.5 Hz where each train was comprised of 4 shocks with an inter shock interval of 10 ms). These results suggest that above 22°C the temperature effects on selected cellular mechanisms in CA1 pyramidal cells is evoked by Schaffer collateral stimulation do not markedly differ in the hamster (a hibernator) and rat (a nonhibernator). Supported by NIA grant NAG 2-331.

**NEURAL PLASTICITY IN ADULT ANIMALS: INDUCED EFFECTS II**

**A COMPUTER ALGORITHM FOR SEPARATING OVERTAPPING SYNAPTIC CONDUCTANCES**

...and T.A. Leach*. Dept. of Surgery, Div. of Neurosurgery, Univ. of Wisconsin, Milwaukee, Wisconsin, U.S.A.

Supported in part by NIH grant NS 34911.

**A BIOLOGICALLY PLAUSIBLE IMPLEMENTATION OF A HEBBIAN COVARIANCE ALGORITHM**

...Division of Neuroscience, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

A Hebbian learning mechanism is one in which synaptic modification is governed by the conjunction or covariance between pre- and postsynaptic activity. Sejnowski (ibid) noted that a Hebbian conjunctive mechanism that explains permanent synaptic enhancement will encounter the problem of runaway instability, due to spurious coincidences, even when pre- and postsynaptic activity are correlated. This runaway instability can be avoided by assuming rapid passive decay of the synaptic enhancement.


Our simulations have suggested ways to construct, from known neurological phenomena, synaptic computations that share some of the desirable features of a covariance mechanism. One solution is just a sum of three identified types of synaptic plasticity—a conjunctive type of synaptic enhancement and two types of synaptic depression. This three-process synaptic mechanism avoids the instability problem and offers a biologically plausible synaptic substrate for certain of the developmental phenomena cited above (Brown, T.H. et al ibid). Our results immediately raise the question as to whether these three forms of plasticity actually co-occur in the same synapses or local circuits. (Supported by the MRC and AFOSR).
334.5


During kindling, the most commonly employed electrographic measure used to assess seizure development is the duration of after-discharge (AD). However, several other electrographic measures can also be quantified.

Male Royal Victorian hooded rats were stimulated and AD recorded through bilaterally implanted bipolar nichrome electrodes in the amygdala. Five different electrographic measures with the following axons were examined: i) duration, ii) number of spikes, iii) amplitude on stimulated side, iv) amplitude on contralateral side, and v) spike frequencies. The five electrographic measures and Racine’s behavioral classification of seizure stage were recorded on each session to give an indication of kindling progression. A multiple regression technique was used to assess the importance of each electrographic measure as a predictor of behavioral stage and the amount of variation which can be accounted for by each electrographic measure.

Results indicate that amplitude on the contralateral side correlates best with the behavioral seizure and can account for approximately 62% of the variation in seizure behavior. Supported by NSERC.

334.7


LTP of the entorhinal cortical (EC)-dentate gyrus (DG) synaptic response correlates with morphological changes at these synapses. Previously we hypothesized that the concave spine synapses represent the population of potentiated synapses. Compared with the control synapses, a set of features in a consistent manner with the potentiated synapses: a post-synaptic concavity at the synaptic interface, bigger spine heads, bigger PSD and membrane apposition surface areas, an increased number of front-line synaptic vesicles, vesicles resting in probability of a polygonal form of the base of the spine. In the absence of stimulation-induced LTP, this same set of synaptic features also distinguishes synapses in the DG so that EC-DG synapses normally potentiate from EC-DG concave spine synapses. For example, the membrane apposition surface area per concave spine synapse is 9.5±0.7 um²/100 um³ while a simple spine synapse has on average 39.6±0.2 um²/100 um³ membrane apposition surface area. Similarly, the mean number of front-line synaptic vesicles along the membrane apposition trace length is 9.3±0.9 for the concave spine synapses and is 4.0±0.4 for the simple spine synapses. Since the post-synaptic concavity correlates with the same set of features whether or not stimulation-induced LTP has occurred, we hypothesize that the concave spine synapses with their set of defining idiosyncratic features are markers for endogenous LTP. If true, the concave spine synapse may identify associative synaptic potentiation, at least for those excitatory axospinous synapses using acidic amino acid transmitter and possessing NMAD receptors, and can be used to identify LTP as a physiological correlate of plasticity.

Supported by NIH NS15488 and NIMH R01 MH4622 to WBL.

334.8

NOVEL MORPHOLOGICAL CHANGES IN HIPPOCAMPAL DENTATE GRANULE CELL PERIKARYA FOLLOWING RECURRENT LIMbic SEIZURES. M.C. Bundman, R.M. Pico, J.Athanikar* and C.M. Call. Departments of Anatomy and Neurobiology and Pharmacology, University of California, Irvine CA 92717.

Recurrent limbic seizures stimulate increased enkephalin synthesis by the dentate gyrus granule cells and lead to changes in the synaptic vesicle populations within their mossy fiber terminal boutons. In the present study, messenger RNA (mRNA) levels were determined in vitro by northern and in situ hybridization techniques. Estimation of the number of these synapses per neuron showed that in kindled rats, however, they were significantly increased (from 3.52 per neuron in controls to 6.05 in the kindled group). The major effect on TH activity was in the central nucleus of amygdala (AC). Met and PPE mRNA levels were also detectable. Alterations in the enzyme or mRNA levels were not detected in the substantia nigra or in the striatum.

Supported by NSF grant BNS 8417098 and RCDA N050015 to CMG.

334.10


Examination of axosomatic synapses in serial sections obtained from the molecular layer of the dentate gyrus has revealed that "perforated" double-headed dendritic spines. Such a spine is attached to a parent dendrite by a single stalk. From the stalk base, multiple axosomatic contacts divide. The present study was designed to elucidate whether such structural synaptic modification is restricted to the terminal field of stimulation. A total of 30-day group of rats were examined. Results indicated that both "perforated" and "nonperforated" synapses were decreased in numbers (by 29 and 30%, respectively) in the MML, compared with control animals; in kindled rats, however, they were significantly increased (by 56 and 51%, respectively). "Perforated" synapses on double-headed spines were quantified in the middle molecular layer of the hippocampal dentate gyrus with the aid of the unbiased stereological disector technique. Estimation of the number of these synapses per neuron showed that in kindled rats, however, they constitute only about 22% of the total population of "perforated" or "nonperforated" synapses in control animals. In kindled rats, however, they were significantly increased in numbers (from 3.52 per neuron in controls to 6.05 in the kindled group). These results support the notion that "perforated" synapses on double-headed dendritic spines represent a structural modification related to enhanced synaptic efficacy.

Supported by Grant BNS-860727 from NSF.
335.1


Sodium channels are responsible for initiating and propagating action potentials in excitable tissue. In the present study, sodium channels were localized on dendrites of identified neurons using two techniques: immunogold labeling and electrophysiological recordings. Immunogold labeling was performed using a polyclonal antibody against sodium channel protein. The antibody was applied to tissue sections, and gold particles were visualized with an electron microscope. Electrophysiological recordings were carried out on identified neurons in brain slices, and potassium currents were recorded to assess sodium channel activity.

335.2

DIRECT IMMUNOGOLD LABELING OF Na+ CHANNEL IMPS IN FROZEN-FRACTURE REPlicas. L. E. Higley, J. A. Johnson, L. E. Dischuk, D. S. Duch, and S. R. Levinson (Depts. of Anatomy and Neurobiology

The distribution of sodium channel immunogold particles (IMPs) on the surface of dendritic spines was analyzed in frozen-fracture replicas of rat brain tissue. The replicas were prepared by freezing the tissue and then fracturing it to expose the surface of dendritic spines. Immunogold particles were detected using a polyclonal antibody against sodium channel protein. The results showed that sodium channel IMPs were concentrated on the plasma membrane of dendritic spines, indicating that sodium channels are crucial components of the dendritic membrane.

335.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF SODIUM CHANNEL SUBTYPES R and R. W. Woolfenden and W. A. Cameron (Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195).

The voltage sensitive sodium channel is a transmembrane protein responsible for the rising phase of the action potential in electrically excitable tissue. Affinity-purified and polyclonal antibodies were used to detect the presence of sodium channel subtypes R and R in rat brain tissue, and nerve terminals. Immunoprecipitation experiments have shown that R and R are expressed primarily in the central nervous system with R predominating in brain and spinal cord and R in dorsal root ganglia. Light microscopic studies have revealed that the overall immunoreactivity for R decreases from rostral to caudal with intense immunoreactivity in the ventral horn and the overall immunoreactivity for R decreases from rostral to caudal with intense immunoreactivity in the ventral horn and spinal cord.
ANTIBODIES AGAINST A CONSERVED INTRACELLULAR SEGMENT OF THE Na+ CHANNEL: Sodium Channel Inactivation. P.M. Vassilev*, T. Scheuer* and W.A. Catterall (Spon: M. Hijmans) Dept. of Pharmacology, SJ-30, University of Washington, School of Medicine, Seattle, WA 98195.

The primary amino acid sequence of the α-subunit of Na channels in some excitable membranes has been defined, but the relationship between structure and function of the channel remains unclarified. The effects of four antibodies, directed against different predicted intracellular segments of the α-subunit, have been tested on whole cell sodium currents in rat skeletal muscle cells. An affinity-purified antibody (anti-SPI9) directed against a highly conserved amino acid sequence in the intracellular segment between domains III and IV (residues 1540 to 1557 of Rgt) induced a substantial slowing of the inactivation kinetics which was blocked by the corresponding peptide. At a holding potential of -110 mV the intracellular application of the antibody increased the time constant of the Na current decay from 2.28 to 4.37 ms during a test pulse to -20 mV. A similar effect was observed at a holding potential of -70 mV, but its onset following seal formation occurred at a three-fold slower rate than at -110 mV. A dependence upon the test pulse potential was also observed. Slowing of inactivation was small for pulses to -50 mV, but was pronounced at more positive test potentials up to 90 mV. Depolarization directed against another predicted sequence in an intracellular segment of the Rgt α-subunit did not significantly affect Na channel inactivation. These results identify a highly conserved intracellular segment between domains III and IV of the α-subunit which may be involved in the channel inactivation mechanism.


We have cloned and constructed a full length cDNA encoding the rat brain Na channel α subunit. The encoded α subunit differs from the Rat2 α subunit of Noda et al (Nature, 335: 991, 1988) at six amino acid positions. It was transcribed in vitro from this cDNA and injected into Xenopus oocytes to produce functional sodium channels. Although the primary sequences of the two α subunits were almost identical, the current-voltage relationship for channels produced from our cDNA was shifted 20-25 mV to the depolarizing direction compared to that reported for the Rat2 subunit (Stuhner et al, Eur. Biophys. J., 14:131, 1987). It is likely that one or a combination of the six variant amino acids is responsible for this shift in the current-voltage relationship. Each of the amino acids has been altered by site directed mutagenesis to the corresponding residue found in the Rat2 α subunit. Voltage clamp analysis of the channels produced from these mutants provides evidence for the location of structural domains in the sodium channel subunit which are important for the voltage dependent channel opening.


The convulsant properties of cocaine are well known and may involve the disinhibition of neuronal pathways within brain. It has been observed that cocaine BCI (COC) and TTX on Na currents by the whole cell patch clamp technique in two cell populations, the muscle neuroblasts ccl4 and tip-e. The effects of TTX and COC on current-voltage relationship for these channels provided evidence for the location of structural domains in the sodium channel subunit which are important for the voltage dependent channel opening.

GATING OF BATRACHOTOXIN-ACTIVATED Na CHANNELS IS ALTERED BY SERRATION VENOM IN PLANAR LIPID BILAYERS. M. O'Leary* and B. E. Kreuger. Dept. of Physiology, Univ. Maryland Sch. of Med. Baltimore, MD 21201.

The effects of batrachotoxin (BTX), Na channels do not inactivate, whereas Na channels that display voltage-dependent block by local anesthetics and tetrodotoxin (TTX) have been found to be more sensitive to Zn than TTX-sensitive subtypes. We compared the effect of Zn and other divalent cations on cardiac and skeletal muscle Na channels. An affinity-purified antibody (anti-SP19) directed against a highly conserved predicted intracellular segment of the α-subunit does not inactivate, however, BTX-activated Na channels, which display slow inactivation kinetics which was blocked by the corresponding peptide. At a holding potential of -110 mV the intracellular application of the antibody increased the time constant of the Na current decay from 2.28 to 4.37 ms during a test pulse to -20 mV. A similar effect was observed at a holding potential of -70 mV, but its onset following seal formation occurred at a three-fold slower rate than at -110 mV. A dependence upon the test pulse potential was also observed. Slowing of inactivation was small for pulses to -50 mV, but was pronounced at more positive test potentials up to 90 mV. Depolarization directed against another predicted sequence in an intracellular segment of the Rgt α-subunit did not significantly affect Na channel inactivation. These results identify a highly conserved intracellular segment between domains III and IV of the α-subunit which may be involved in the channel inactivation mechanism.


The divalent cation, Zn, has been implicated as a subtype-specific inhibitory modulator of glutamate-activated channels in mammalian brain. In what may be an analogous situation, certain Na-channel subtypes may be insensitive to local anesthetics (TTX) but have been found to be more sensitive to Zn than TTX-sensitive subtypes. We compared the effect of Zn and other divalent cations on heart and skeletal muscle Na channels. The following values were obtained for apparent blocking Kd's (in mM) at 0 mV and symmetrical 0.2 M NaCl, pH 7.4: Mg (48), Ca (9), Sr (2), Ba (88), Mn (18), Co (13), Ni (11) for muscle and Mg (51), Ca (41), Sr (75), Ba (76), Mn (20), Co (15), Ni (13) for heart. In contrast to the similar affinity of these cations for two subtypes, Zn blocked the muscle subtype with a low affinity (Kd ≈ 11 mM) and the heart subtype with a 120-fold higher affinity (Kd = 0.09 mM) at 0 mV. In contrast to a fast block by Zn for the muscle channel, Zn induced brief closing events in the heart channel with a mean duration of about 20 ms from -60 to -600 mV. The frequency of Zn-induced blocking events was both concentration and voltage dependent, indicating a voltage-dependent association rate for Zn. (NIH AR38796)
335.11
PRESENCE AND SIGNIFICANCE OF TETRODOTOXIN SENSITIVE SODIUM CHANNELS IN CAROTID BODY CELLS. A. Roche¹, A. Obejo², C. Gonzales and B. Herrera³ (SPON: M. Rodrigo Angulo). Dept. Biologia, Facultad de Medicina. Universidad de Valladolid. 47005-Valladolid (Spain).

There are clues that depolarization of chemoreceptor cells (c.b.) is mediated by sodium channels which can be inhibited by local anesthetics. The present study was designed to test the hypothesis that these sodium channels exist in the carotid body, and to test the possibility that they might contribute to the secretory response when stimulated by low PO2. We explored the presence of type 1 cells of tetrodotoxin in (TTX) or veratridine Na+ channels by exposing 100 µM Na+-free saline solutions at different PO2 tensions or with veratridine either with or without TTX. We found: 1) Veratridine evokes Na+-DA loaded c.b. were superfused with saline solutions at different PO2 tensions or with veratridine either with or without TTX. We found: 1) Veratridine-evoked release was dependent on Na+ and Ca++- 2) Veratridine-evoked release was dependent on Na+ and Ca++. 3) Veratridine (30 µM) evoked release was blocked by TTX (1 µM). 4) Low PO2 (0-40 torr) evoked release was partially inhibited by TTX (1 µM), but the inhibition was not as complete as in the case of high PO2. These findings indicate that chemoreceptor cells possess Na+ channels and their involvement in the physiological stimulus-secretion reaction.

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335.13
INDUCTION OF STX-SENSITIVE SODIUM CHANNELS IN CULTURED ASTROCYTES. P.J. Yarovsky, D.S. Brougher,∗ and B.K. Kroeger. Deps. of Pharmacology & Experimental Therapeutics and Physiology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201

Tracer flux and STX binding studies reveal a progressive change in the STX affinity of cultured neonatal rat astrocytes. Low STX affinity Na channels (Kg = 40 nM) were present throughout the 1st week in culture. High STX affinity Na channels (Kg = 0.4 nM) were present at very low levels during the first week, but increased rapidly over the next 5 days reaching a maximum (2.2 pmol/mg protein) by the end of the second week. This spontaneous change in STX affinity was coincident with changes in cellular morphology during the initial 7 inducible and cell-surface compartments with few processes to stare cells with numerous processes. Replacing standard medium (MEM = 10% FCS) with serum-free medium containing 2% NCS on day 7 induced a morphological differentiation within 15 hr in all cells. This change in medium also rapidly increased the proportion of high STX affinity Na channels to 67% at a time when the retina normally consisted only 5%. Although serum-free medium promotes the appearance of oligodendrocytes, we found no sustained STX binding to serum-free cultures of oligodendrocytes. Since low STX affinity channels were still present in these chemically defined cultures, both high and low STX affinity Na channels probably exist in mature stellate astrocytes. (Support: NIH and NSF)

335.14

Sympathetic deafferentation of the hippocampal pyramidal cells (HPC) is thought to evoke Na+ dependent spike discharge at both the axon hillock and one or more dendritic sites containing a high density of Na+ channels. The present study examined the site of origin of stratum radiatum (SR) evoked spike discharge in the in vitro hippocampal slice preparation. HPC afferents and dendritic spike discharge was examined by testing the sensitivity of intra- and extracellular potentials recorded in the cell body layer (axon hillock) and proximal apical dendrites to local application of TTX (40 µM). At very high stimulus intensities SR stimulation evoked a fast negative population discharge in proximal dendrites with shorter peak latency than the cell body population spike. TTX ejection to the axonic region reduced the cell body population spike with little change in the proximal dendritic negativity. In contrast TTX restricted to proximal dendrites reduced both the dendritic and cell body population discharge. At threshold, SR-evoked proximal intradendritic spikes were blocked in an all-or-none manner by TTX ejection to the cell body layer. At higher intensities the same ejection abolished the cell body population spike but reduced the intradendritic spike by <20%. Blockade of the intradendritic spike occurred subsequent to diffusion of TTX to the proximal dendrites. The results suggest that at threshold SR-evoked spikes discharge originates in or near the cell body layer. Higher intensities of activation shift the site for spike initiation from the cell body layer into the proximal apical dendritic region.

335.15

Voltage-dependent sodium channels are permeable to a number of small organic cations including guanidinium ions. Indeed, a recent report (Biol. J. Pharmacol. 12(1986)291) suggested similarities between [14C]guanidinium ion and [3H]Na ion influx into crude synaptosomes from rat brains. In the present study [14C]guanidinium ion influx into purified synaptosomes from mouse cerebral cortex was characterized in more detail than tetrodotoxin (TTX) and nictitating membrane to either pre- or post-ganglionic stimulation. In a rat cortical synaptosome preparation, B-120 (100 µm), blocked both potassium-stimulated and basal calcium flux by 75%. This regard, B-120 was equipotent to verapamil. B-120 has been previously reported to reduce organophosphate toxicity (J. Med. Chem. 31:807, 1988) in mice and guinea pigs. It seems likely that the organophosphate protection seen with B-120, as well as its hypertensive effects, may be related to its capacity to block calcium channels.

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335.16
LOCAL ANESTHETICS: INHIBITORY EFFECTS ON BATRACHOTOXIN-ELICITED SODIUM FLUX AND PHOSPHODIESTERASE BREAKDOWN IN GUINEA PIG CEREBRAL CORTICAL SYNAPTONES. Y. Nishiawa*, F. Watanabe and T.W. Daly. Lab. of Bioorganic Chemistry, NIDEK, NIH, Bethesda, MD 20892

Local anesthetics inhibit the sodium influx and the inositol phosphate accumulation elicited by the sodium-channel activator batrachotoxin in guinea pig cerebral cortical synaptosomes. Inhibitory effects of local anesthetics on sodium influx correlate closely with inhibitory effects on binding of a tritiated batrachotoxin analog to sodium channels in rat brain synaptosomes. Inhibitory effects of local anesthetics on sodium influx also correlated with inhibitory effects on inositol phosphate accumulation. Bufrocin, evipacrine, lidocaine, and certain analogs of TTX had little if any effect on inhibition of sodium influx and inositol phosphate accumulation. Local anesthetics also inhibit inositol phosphate accumulation that was induced by carbamylcholine, which activates two types of synaptic transmission, a tetrodotoxin-sensitive and a tetrodotoxin-insensitive pathway. Certain local anesthetics, such as dibucaine, inhibited the tetrodotoxin-sensitive pathway with higher potency than for the tetrodotoxin-insensitive pathway. Other local anesthetics, such as quinacrine inhibited tetrodotoxin-sensitive and insensitive pathways with equal potency. The data support an involvement of sodium channels in regulating phosphodiesterase breakdown in synaptosomes, but indicate that effects of certain local anesthetics on muscarinic receptors or on the phospholipase C system may complicate interpretations.
PHOSPHODIESTERASE HYDROLYSIS INDUCED BY SODIUM CHANNEL ACTIVATORS IN HOUSE SPIDER'S ROLE OF NA, Ca, AND Mg IN HABITUATION


Veratridine (VERA) and Ca++ channel activators are known to increase phosphorylation of PI (Pi) hydrolysis in brain. The present work with mouse cerebral cortical, glial, and sympathetic neurons shows (1) the entry of Na+, (2) depolarization, and (3) Ca++ influx through voltage-dependent Ca++ channels. Release of neurotransmitters is known to be supported by increased Ca++ influx. Depolarization by 30 mECl enhanced PI hydrolysis. This response was not blocked by tetrodotoxin (TTX) and not stimulated by Ca++ channel agonists (10 nM Bay K 8644) or prevented by Ca-antagonists (10 nM nifedipine or 10 nM nifedipine), indicating the lack of involvement of Na and Ca++ channels in the ECL effect. The PI response to VERA (10 nM) was blocked by TTX but not affected by the Ca- agents. The PI response to KCl was not additive with that to VERA or TTX, suggesting a nonspecific response. This could be a modulation of intracellular Na levels, in the case of ECl by means other than Ca++ channels. Consonant with this possibility is the increase in PI hydrolysis found with 3 mM monensin promoting Na influx without inducing depolarization, and the lack of the PI response to 30 mECl, 10 nM VERA, and 10 nM G4/12 of SOV in sodium-free media. In the latter case, a positive response to 2 mM carbamol demonstrated the functional activity of the PI system. These results suggest a direct role for Na in regulating PI turnover. (DA 03025)

MODULATION BY VERATRIDINE, BATRACHOTOXIN AND MONENSIN OF PHOSPHODIESTERASE HYDROLYSIS IN CEREBROCORTICAL SLICES

Elizabeths Hospital, Wash. D.C. 20032.

The contractile protozoa, Stentor, habituates during repeated mechanical stimulation due to a progressive decrement in receptor potential amplitude. Repetitive elicitation of action potentials and contractions is required to maintain this decrement; repetitive elicitation of receptor potentials alone produces no decrement. After responding to the first 3 to 10 stimuli, an occasional animal ceased contracting despite continued elicitation of receptor and action potentials. In these cases the partially decremental receptor potential recovered to prestimulus levels. To further study the contractile mechanism an intracellular signal which modifies the mechanoreceptor channels and produces habituation.

An increase in intracellular Ca++ is not the signal for habituation since receptor potentials decrement normally when recorded with 50 mM EGTA-filled cells present Ca++-dependent inactivation of the voltage-dependent Ca++ channels. Different types of drugs, toxins and ions known to modify the efficacy of second messenger systems were screened for their ability to alter habituation in Stentor. Only phosphodiesterase inhibitors and dibutryl cAMP increased the animal's rate of habituation without affecting their sensitivity to photic, electrical or spaced mechanical stimuli. Therefore, cAMP may serve as a second messenger in the production of habituation in Stentor.
336.3 ASSOCIATIVE LEARNING IN THE LEech: BEHAVIORAL AND CELLULAR EFFECTS OF PREDETLYABILITY OF OCCLUDER NEURONS.  Chien L. Shih, Dep. of Biology, Yale Univ, New Haven, CT 06511.

The pairing and the predictive relationships between stimuli have been shown to be critical variables in associative learning for both vertebrate and invertebrate species. We previously addressed the pairing dependency in the interneurt. In this study we used a semi-chronic preparation to address both of these variables and their cellular correlates.

Our behavioral procedures were similar to those originally used to study contingencies of invertebrates (Roesler, et al.). We compare the learning performance of leeches in four groups. Leeches in Group (Gp) 1 received 30 CS-US pairings (touch-shock). This procedure resulted in a significant increase of lat. tailflip escape probability of later lat. giant mediated tailflip escape. Gp 1 and 2 were matched for US frequency. The additional USs degraded the predictive CS-US relationship. Leeches in this group show significantly less learning. Since leeches in Gp 2 received more USs than leeches in the paired US (Gp), a third Gp was run which received 42 paired CS-US presentations, thus controlling the total number of US presentations. Leeches in Gp 3 showed learning comparable to the learning observed in Gp 1. Finally, leeches in Gp 4 received 30 random presentations of the CS and US. Leeches in this group showed a decrement in behavior.

Cellular experiments demonstrate that the Rensius cell (R) may be implicated in this phenomenon. This, the R cell fires in response to the US and, the firing decreases to repeated CS-alone presentations. No decrement has been observed during pairings. The decrement may be mediated through autoreceptors on the Rensius cell.


Previous work has shown that habituation of the Triton giant escape swim involves a decrease in the number of cycles per swim. We now report that habituation is associated with changes in three additional aspects of the swim: (1) non-motor correlated intertrial interval (NMI) duration (1st trial); (2) latency (1st trial) to a 7% escape threshold; (3) number of trials to a 7% escape threshold.

In addition, the velocity of the tailflip increased from 6.3 to 8.0 m/s (N=7, p<0.01). The maximum firing frequency of dorsal motor neurons, which control the flexion movement, decreased (N=6, p<0.01) in isolated brains. The suppressibility of these behaviors, decreased from 34% in the intact animal to 14% in isolated brains. The similarities between behavioral and isolated brain responses suggest that central nervous system modifications are partially responsible for habituation in Triton.

To test for a behavioral threshold, six animals were simulated at 2 min intervals. After 20 stimuli, all animals failed to swim a response. In vitro responses were isolated for 30 sec to two stimuli. The swim motor program was elicited 10 times (2 min ITI) in isolated brains by electrical stimulation of a nerve root. The duration of the swim was measured by the period of interneuron C2 bursts, increased from 6.2 to 8.0 sec (N=7, p<0.01). The maximum firing frequency of dorsal motor neurons, which control the flexion movement, decreased (N=6, p<0.01) in isolated brains. The similarities between behavioral and isolated brain responses suggest that central nervous system modifications are partially responsible for habituation in Triton.

Recent studies indicate that multiple circuit modifications may mediate habituation in Triton (Frost, W.N. and P.A. Gerttula, Soc. Neurosci. Abatr., 1987). We intend to explore the relationship between the multiple circuit modifications and the multiple behavioral changes reported here. Supported by NS17225 and NS07247.


Long-term facilitation (LTF) at the crayfish neuromuscular junction is induced by ketamine stimulation and persists for hours. Induction of LTF has been attributed to ionic imbalances produced by the stimulation. The membrane potential changes in Na+ and Ca++ recover shortly after stimulation, yet facilitation persists for hours. The present study investigates second messenger involvement in the induction of LTF. Activators of PKC (CAMP, IMX and Forskolin) are effective in producing a long-lasting facilitation similar to the second phase of LTF. L2X22 (a pre-synaptic inhibitor of PKC) blocks the second phase of LTF at synapses near the injection site, whereas normal LTF develops at synapses distant from the injection site within the same preparation. Localisation of PKC was confirmed by fluorescent tagging of the PKC. Adenylate cyclase activity in LTF injection site is further supported by results using SQ22,536, an AC inhibitor. Localised injection of SQ22,536 blocks the second phase of LTF near the injection site, while synapses distant from the injection show normal LTF. These experiments establish a role for AC activation in producing LTF. Supported by MRC Canada and GSF scholarships.

336.6 INTRACELLULAR CORRELATES OF RESTRAINT-INDUCED MODULATION OF THE CRAYFISH LATERAL GIANT ESCAPE RESPONSE. E.L. Wu and E.B. Kram. Dep. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Restraint suppresses the lateral giant (LG)-mediated tailflip escape reflex of the crayfish. This suppression involves a tonic form of inhibition descending from throracic and higher levels of the crayfish nervous system. Recent studies indicate that multiple circuit modifications may mediate habituation in Triton (Frost, W.N. and P.A. Gerttula, Soc. Neurosci. Abatr., 1987). We intend to explore the relationship between the multiple circuit modifications and the multiple behavioral changes reported here. Supported by NS17225 and NS07247.

336.7 OCTOPAMINE MODULATION OF THE SENSORY SYNAPSE IN CRAYFISH LATERAL GIANT ESCAPE RESPONSE CIRCUIT. J. Butteman and E.B. Kram. Department of Psychology, University of California, Los Angeles, CA 90024.

Traumatic events cause a prolonged increase in the probability of a lateral giant mediated tailflip escape responses to sudden abdominal stimuli. This behavioral sensitization is associated with increased stimulus sensitivity and delayed onset of the tailflip reflex circuit. Octopamine mimics these effects.

Here we studied the effect of octopamine on the intra-cellular calcium concentration and the membrane potential of the tailflip reflex circuit. Octopamine mimics these effects.

Here we studied the effect of octopamine on the intra-cellular calcium concentration and the membrane potential of the tailflip reflex circuit. Octopamine mimics these effects.


We investigated the probability that neuronal proteins of A. californica were altered by procedures that mimic those used to produce long-term sensitization. Samples from 20-gel electrophoresis of the tailflip reflex circuit. Octopamine mimics these effects. We investigated the probability that neuronal proteins of A. californica were altered by procedures that mimic those used to produce long-term sensitization. Samples from 20-gel electrophoresis of the tailflip reflex circuit. Octopamine mimics these effects.
Evidence from Drosophila and Aplysia implicates persistent activation of the cAMP cascade in memory. Different processes in the cAMP cascade may contribute to different aspects of the long-term memory. One such process is autophosphorylation of cAMP-dependent protein kinase (PKA), in which the catalytic subunits (C) phosphorylate the regulatory subunits (R). We developed a quantitative model for the interaction between the two PKA subunits, making use of experimentally defined kinetic rate constants and assuming eight functional states for R: four involving dephosphorylated R (RC, R-CAMP-C, R-CAMP-R, and R-CAMP-D) and four states involving phosphorylated R. Computer simulations showed that following a transient (several seconds) rise in intracellular cAMP levels, PKA can be activated for tens of minutes under appropriate physiological conditions. This long-term activation of the protein kinase is due in part to the effects of phosphorylation of the regulatory subunit, which decreases the affinity of the regulatory subunit for the catalytic subunits. We used this model to simulate the activation of Protein Phosphatase 2A (PP2A) in Drosophila learning and memory mutants, with primary or secondary defects in the cAMP cascade. Our model predicts defective kinetics of PKA in C. elegans, in Caenorhabditis elegans, in Aplysia, and possibly in other organisms. A) PKA is central to sensitization, habituation, and classical conditioning, and B) PKA may retain information in short-term memory by the process of long-term activation. (Supported by the US-Israel Binational Science Foundation, Jerusalem.)

336.12 PROTEIN KINASE C INHIBITION PREVENTS SHORT-TERM BUT NOT LONG-TERM LIGHT-5-HT-INDUCED ENHANCEMENT OF GENERATOR POTENTIALS IN HERMISSENDA B-PHOTORECEPTORS. J. Forrester* and T. Crow (SPON: A. Molter). Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77025.

Light paired with direct application of 5-HT to the exposed nervous system of otherwise intact Hermisenda produces short- and long-term changes in the amplitude of light-evoked generator potentials recorded from identified photoreceptors. Previous results have suggested that activation of protein kinase C (PKC) may contribute to the induction of plasticity produced by pairing light and 5-HT. We now present evidence that an inhibitor of PKC produces different effects on short- and long-term plasticity. Application of the PKC inhibitor H-7 (1 μM) (Hidaka, 1984) blocked the short-term enhancement of light-evoked generator potentials produced by light and 5-HT (mean peak amplitude=41.4mV, mean amplitude at light offset=27.8mV; H-7 mean peak amplitude=39.2mV, mean amplitude at light offset=26.8mV). Light responses following the application of 5-HT without H-7 were enhanced (mean peak=43.6mV, mean amplitude at light offset=29.7mV). In contrast to the short-term effects, H-7 did not block the long-term (24 hrs) enhancement of generator potentials produced by pairing light and 5-HT (mean peak amplitude=43.3mV, mean amplitude at light offset=29.8mV). These results suggest that other messenger systems may contribute to the long-term enhancement of light responses produced by light and 5-HT.
336.15 MODIFIABLE BEHAVIOR AND AGE-SENSITIVITY MAY BE RELATED IN APYLIA. B. T. Lancaster*, Dep. Physiol. & Biophys., Univ. of Ky., Lexington, KY 40536. Peretz et al. (1984) proposed that the modifiable gill withdrawal reflex (GWR) is age-sensitive to the periodic respiratory gill pumping movements (GPM). We based this on the age-impaired function of neuron L7 and the age-relevant function of LDG1, LG5 and LDG2 are major contributors of the GWR and GPM respectively. To determine the applicability of this proposal to freely moving animals we examined the GWR and GPM in the same age range (ca 150 days) and old (ca 250 days) animals. The GWR was elicited by artificial seawater (ASW) jets of 0.4 to 8.0 g/cm². The GPM duration was reduced in old animals. F(4,96) = 2.51, p<0.05. The GWR habituated faster in old than in mature animals to repetitive jets of 2.0 g/cm², F(9,198) = 2.74, p<0.005. Elevated GPM rates were elicited by placing the animals in ASW from 7.8 (normal ASW) to 3.0 for 5 min intervals. The GPM rates were the same in both groups. The relationships between the GWR and GPM were significant. Rather, they can be regulated by modulatory transmitters, such as 5-HT, and by competitive interactions with the processes of other sensory neurons. Application of 5-HT to sensorimotor cultures causes growth of the sensory processes, particularly those contacting L7's major neurite. Also, in mature cell cultures different neurites of L7 appear to be occupied by processes from different sensory neurons. Only the major neurite of L7 is regularly colonized by processes from different sensory neurons and, in these instances, the different processes are segregated onto different regions of the neurite. These findings provide structural correlates for the functional plasticity which characterizes this synapse, as well as evidence for a previously unsuspected competitive mechanism regulating the formation of connections between Aplysia sensory and motor neurons.

336.16 PERSISTENCE OF ULTRASTRUCTURAL CHANGES AT IDENTIFIED SENSORY NEURON SYNAPSES DURING LONG-TERM SENSITIZATION IN APYLIA. M. Chen and C.H. Bailey. Ctr. for Neurobiol. & Behav., Dept. of Anat. and Cell Biol., Neuro. & Psych., Columbia P.S., and NYSPI, NY, 14032. The time course of changes in active zone morphology at identified sensory neuron synapses was examined at different intervals following long-term sensitization of the gill-withdrawal reflex. HRP-labeled varicosities in 20μm slab-thick sections were re-embedded, thin sectioned, and analyzed through a blind procedure. 693 varicose neuron varicosities taken from 18 animals were treated in this fashion. As reported in an earlier study, we have found that long-term sensitized animals examined within 48 hrs following the completion of training demonstrates no increase in the incidence (0.395 ± 0.03 S.E.M., vs. 0.21 ± 0.02) length (0.51μm ± 0.02 vs. 0.29μm ± 0.01) and vescicle complement (4.2 ± 0.19 vs. 1.43 ± 0.13) of sensory neuron active zones compared to control animals. The increase in active zone number is maintained at 1 week (0.386 ± 0.02, N=3 vs. 0.22 ± 0.02, N=2; t=1.9, p<.05) and is only partially reversed at the end of three weeks (0.27 ± 0.01, N=3 vs. 0.19 ± 0.01, N=2). In contrast, the increase in active zone size and vescicle complement is not present after 24-48 hrs. The relative permanence of changes in active zone number and their similarity in time course to both changes in variability number as well as the duration of the memory strengthens the evidence that alterations in the number of sensory neuron synapses may contribute to the retention of long-term sensitization.

336.17 MORPHOLOGICAL EVIDENCE THAT COMPETITION FOR POSTSYNAPTIC SPACE AND SEROTONIN STIMULATION CAN REGULATE THE GROWTH OF APYLIA SENSORY NEURONS IN CELL CULTURE. D. L. Glanzman, E.R. Kandel and S. Schacher. Howard Hughes Med. Instit., Ctr. for Neurobiol. & Behav., Columbia P.S., and NYSPI, New York, NY. (1982). We have examined the relationship between morphological changes and long-term synaptic changes for Aplysia sensorimotor synapses in culture. After assessing the strengths of synaptic connections between sensory neurons and motor cell L7 electrophysiologically, the processes of the sensory neurons were visualized via fluorescence microscopy. Our findings suggest that the neurites which mediate synaptic contact between a sensory neuron and its postsynaptic target are not "hard-wired." Rather, they can be regulated by modulatory transmitters, such as 5-HT, and by competitive interactions with the processes of other sensory neurons. Application of 5-HT to sensorimotor cultures causes growth of the sensory processes, particularly those contacting L7's major neurite. Also, in mature cell cultures different neurites of L7 appear to be occupied by processes from different sensory neurons. Only the major neurite of L7 is regularly colonized by processes from different sensory neurons and, in these instances, the different processes are segregated onto different regions of the neurite. These findings provide structural correlates for the functional plasticity which characterizes this synapse, as well as evidence for a previously unsuspected competitive mechanism regulating the formation of connections between Aplysia sensory and motor neurons.

337.1 NEW APPARATUS FOR OPTICAL RECORDING OF NEURON ACTIVITY. J.-Y. Wu*, T. Fantani*, N. Abdellaziz*, L.B. Cohen, D.M. Sinesman, D. Schlimovich, A.L. Cohen, C. Xiao*, H.-P. Hoppe*, and J.A. London. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT. Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX, 78285. In an attempt to facilitate the implementation and increase the usefulness of optical recording methods, we are in the process of introducing a new photodiode array, associated amplifiers, and computer data acquisition, made by Centronics Ltd., has 464 elements (instead of the present 144) to provide better spatial resolution. The amplifier is to be similar in electronic characteristics to those presently in use but would be made with either surface mount or hybrid technology (hence relatively less expensive). The new computer system is a 32 bit computer using the VME bus and a 68020 CPU. This Motorola, VERSADOS system is also being considered. While we expect that will be an improvement on the old, the main limitation on the optical recording methods will still be the rate at which optical signals from voltage or ion sensitive dyes.

337.2 SMALL NETWORKS OF ADAPTIVE ELEMENTS THAT REFLECT THE PROPERTIES OF NEURONS IN APYLIA EXHIBIT HIGHER- ORDER FEATURES OF CLASSICAL CONDITIONING. L.H. Byrne, D. Buonomano*, J. Corcos*, S. Patel* & D.A. Baxter. Dept. of Neurobiol. & Anat., The Univ. of Tex. Med. Sch., Houston, TX 77225. Previously, we developed a single-cell mathematical model of the sensory neurons in Aplysia (Gingrich & Byrne, J. Neurophysiol 53:652, 1985; J Neurophysiol 57:1705, 1987). This single-cell model accurately simulated many aspects of empirically observed neuronal plasticity that are to be cellular correlates of simple forms of nonassociative and associative learning. The present study extends our analysis by incorporating this single-cell model into a simple network of elements which reflect the neuronal properties and connectivity patterns in Aplysia. An initial network contained two sensory neurons (SNs) both of which excited a single facilitatory interneuron which both feed back onto the SNs. An assumed property of the FN was that its output was activated by a result of its activation (Hawkins & Kandel, Psychol Rev 91:375, 1984). Simulations from this network exhibited 2nd order conditioning but no asymptomatic blocking when the original model of the SN was used. Both 2nd order conditioning and asymptomatic blocking were simulated by modifying the model such that: 1) the synaptic strength of the conditioned SN (CS- cell) was at least twice as large as an unconditioned SN (CS+ cell), 2) the time required for complete accommodation of the FN was less than the minimum ISI necessary for conditioning in the SNs, and 3) the CS+ levels within the SNs first must surpass a threshold value before associative plasticity can occur. We also investigated how the incorporation of additional interneurons that receive excitatory input from, and feed back to inhibit the SNs, would alter the above parsimony and possibly make the network more robust. Several models proved functional, but each required specific assumptions. Supported by grant AFOSR 87-620.
337.5 DEVELOPMENTAL DISSOCIATION OF DISHABITUATION AND SENSITIZATION IN THE TAIL-WITHDRAWAL REFLEX OF APLYSIA. D. Buonomano*. J. Nevrosci.

In conclusion, dishabituation and sensitization of tail withdrawal can be developmentally dissociated in juvenile Aplysia. Since the tail-withdrawal reflex is well suited for a cellular analysis (Walters et al., 1983), it will be of interest to explore the ontogeny of dishabituation and sensitization in this system on a mechanistic level.

337.6 DEVELOPMENT OF TAIL SENSORY NEURONS IN THE PLEURAL GANGLIA OF APLYSIA. L.J. Cleary, M. Stopfer, and T.J. Carew. Dept. of Biol. and Psych., Yale Univ., New Haven, CT 06520

Tail sensory neurons in the VC cluster of the pleural ganglion of Aplysia are important sites of plasticity underlying different forms of learning in the tail-withdrawal reflex (Walters et al., 1983; Scholz and Byrne, 1987). Stopfer and Carew (1988) have recently shown that dishabituation and sensitization emerge differentially in this reflex. A first step in analyzing the cellular mechanisms underlying this developmental dissociation is to define and characterize the tail sensory neurons in juvenile animals.

To examine the development of tail sensory neurons, injection-pulse recordings (which contains axons of tail sensory and motor neurons) with Ni**+-lysine in 3 juvenile stages (1), E12, and L12) and 3 early adult substages (13A, 13B, and 13C). As early as stage 11, strong cell bodies appeared in an identifiable cluster. We found that there was no increase in the number of filled cells during juvenile development (Stage 11, 2 number of cells: 10S; Stage 13C, 212S). However, there was a significant increase in cell diameter (F=190, p<0.001), comparing 1 to 4 μm in Stage 11 to 5 to 42 μm in Stage 13C. This increase in mean diameter reflected a selective increase in the number of large cells, and a concomitant decrease in the number of small cells, resulting in a transition from a unimodal distribution of cell sizes in Stage 11, to a bimodal distribution of small and large cells in Stage 13C. The gradual nature of this transition and the lack of an increase in total cell number suggests that the emergence of the suprapopulation of large cells in Stage 13C is due to the growth of previously existing small cells.

Having identified a candidate population of tail sensory neurons early in development, we are now characterizing their biophysical properties to examine the development of neuromodulation in the tail withdrawal circuit.

337.7 IMMUNOBOTY TRANSMISSION PRODUCES HETEROSTROPHIC INHIBITION OF THE SENSORY-MOTOR CONNECTION MEETING THE TAIL WITHDRAWAL REFLEX OF APLYSIA. D. Stopher, G. Wroth, S. J. L. Buxton, S. J. J. Buxton, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77025.

The mechanisms within sensory neurons contributing to sensitization of defensive reflexes in Aplysia can be activated by the neurotransmitter serotonin. Varicosities containing serotonin are distributed among the cell bodies of sensory neurons in the pleural ganglion. To further characterize the morphology of serotonin varicosities, we have identified serotonin varicosities in Stage 11 animals. To identify serotonin varicosities, we used the immunoperoxidase technique (Eldred et al., 1983) with correlated light (LM) and electron microscopy (EM).

In the LM, we observed numerous varicosities surrounding somata of sensory neurons. In favorable sections, labeling of fibers surrounding the axon hillock could also be observed. Varicosities (n=1) were classified into three groups based on size: small (less than 5 μm), intermediate (5 to 20 μm), and large (20 to 50 μm). Individual axons can form varicosities of all three types, but branch only at those of large size. Because the somata of sensory neurons are encapsulated by glia, it is necessary to confirm that these varicosities contain serotoninergic varicosities. To do this, we performed several control experiments. First, we injected animals with serotonin (which naturally occurs in Aplysia) to confirm that these varicosities contain serotoninergic varicosities.

In conclusion, serotoninergic varicosities may make apparent synaptic contacts with motor neurons and provide a connection for the transmission of serotoninergic varicosities. These results suggest that under the appropriate physiological conditions, serotoninergic varicosities may play a role in the inhibition of defensive reflexes in Aplysia.

Noxious stimuli such as tail shock produce transient inhibition as well as longer-lasting sensitization of the Aplysia gill- and siphon-withdrawal reflex. A specific type of presynaptic inhibition of the siphon sensory neurons is elicited by the combined FMRFamide immunofluorescence with a fluorescent dye backfilling from the abdominal ganglion (the location of the siphon sensory cell). This technique reveals a single cell in the left pleural ganglion, which we have named LPL16. LPL16 is excited by strong tactile stimulation to the entire body surface, and fires a phasic burst of spikes in response to tail shock. Intracellular stimulation of LPL16 with a similar burst produces inhibition of the siphon sensory neuron to a motor neuron giga seal (average decrease = 32%, p < .01). This stimulation also produces lowering of the action potential in the neuron in the presence of 100 mM TEA (average decrease = 26%, p < .01), indicating that the inhibition is in part presynaptic.

These and previous results show that tail shock elicits two populations of modulatory neurons, including serotoninergic facilitatory neurons (CS1) and FMRFamide inhibitory neurons (LPL16). Further characterization of these neurons may help elucidate the organization of neuromodulation in Aplysia.

337.14 GABA MEDIATION OF VISUAL-VESTIBULAR INTERACTION IN HEMISSENNDA ML. Andrews* and D.L. Allen, UMCN, NINCDS-NIH Bethesda, MD 20892.

Convergence of DS and UV pathways activated during associative conditioning of the nodubranch mullus Hemisemna involves inhibition of type B photoreceptors by hair cells caudally located in statocysts. This inhibition, manifest as IPS by type B cell stimulation of presynaptic hair cells, was unaffected by the bath application of agents known to act as either agonists or antagonists of identified neurotransmitters (NE, ACh, etc.) (Langenes et al., 1987). 

Now it is known that the GABA antagonist bicuculline MeBr consistently blocked type B cell hyperpolarization induced by hair cell impulses. GABA and the GABA-A antagonist bicuculline MeBr blocked both IPSPs and EPSPs in the synaptic input to central gill motor neurons. In addition these peptides reduced the efficacy of the central gill motor neurons to produce gill withdrawal response. Finally, conopressin G and 5, greatly increase the frequency of spontaneous gill movements.

Hermesnida is able to store predictive relationships between events if they occur in a temporal context. This type of associative learning may be mediated by cellular interactions between neurons and be stored by neuromodulation of certain ionic channels. A quantitative model simulated on a computer model of the hypertenous levand and built to relate the temporal specificity at the behavioral level to the underlying biophysical mechanisms at cellular and molecular levels. The network consists of sensory neurons (photoreceptors and haircells), convergence sites on photoreceptors and motor neurons. Seven membrane conductances were modeled by Hodgkin-Huxley-like equations to predict the receptor potential and excitability of Type B photoreceptors (neuronal calcium was considered) by considering light-induced inactivates release, voltage-gated influx, buffer, diffusion and pumps. Haircells fire with reorientation and indirectly cause calcium influx on B-photoreceptors through exocytotic synapses. Phorocytosis is induced to clear PIP2, thereby increasing diacylglycerol (DG). The excitability of the convergent sites, located on the photoreceptor somata and the post-synaptic region on the axon, is modulated by the activation of protein kinase C, which depends on the elevation of calcium and DG. The model predicts that repetitive association of a stimulus (CS) and reaction (US) with 1 second delay will optimally increase the excitability of B-photoreceptor, which eventually can excite the motor neurons involved in foot extension in absence of haircell inputs.


Previous research has shown that GABAergic antagonists drugs do not reverse recovery from sensorimotor lesions following unilateral cortical lesions (Watson and Kennard, 1945; Brailowsky et al., 1986; Schallert et al., 1986). However, the excitant effects of these drugs are due to the enhancement of GABAergic function or to their anticonvulsant properties has not yet been determined. In the present experiment we investigate the effects of MK-801, an anticonvulsant agent that is noncompetitive antagonist at the N-Methyl-D-aspartate (NMDA) receptor (Cline et al., 1982; Kemp et al., 1986; Wong et al., 1986), on recovery of behavioral function following unilateral cortical ablation. Results demonstrate that rats with brain ablation in either the frontal medial (FMC) or sensorimotor cortex (SMC) and received either MK-801 (1 mg/kg) or saline injection beginning 12-16 hrs after surgery. Subsequent experiments were given on days 2, 4 and 6. Behavioral tests measured sensorimotor asymmetries (i.e. a bilateral-tactile stimulation test) and forelimb placing. Unlike other anticonvulsant agents, MK-801 did not reverse recovery. Indeed, results from rats sustaining PMC lesions suggested that MK-801 administration from sensorimotor asymmetry (p<.05 for group; days; and the group X days interaction). A similar trend was found in rats with SMC lesions. An independent analysis of activity indicated that unlike GABAergic drugs (i.e. diazepam, MK-801) increased the percentage of time that the rats spent moving. These results are consistent with the view that the retardation of recovery observed after sensorimotor ablation may be due to the specific enhancement of GABAergic function. Supported by NIH grant NS-23964 awarded to T. Schallert.


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Electrophysiological recording has demonstrated a one-to-one correspondence between vibrissal follicles and barrels in mouse S1 cortex. We have reported on a mapping study using carbon fiber electrodes lowered into the S1 barrel cortex under halothane anesthesia. Penetrations were at 100 µm intervals. Posterior follicles of the Crown were denervated (Melzer et al., the Vol). Maps of the barrel cortex contralateral to the lesion were made at 4 in. 16, 18, 24, 32 and 64 in. 2 days after the lesion (p), as well as from control animals. After recording, whiskerpads and brains were processed for histology. At 16 and 32 days p.l. two types of maps could be distinctively distinguished: a) The Control Group: i.e. no responses detected nor the representations of whiskers of rows B and D were enlarged, as were the receptive fields at individual penetrations; moreover, responses of affected follicles were obtained. The map of the 4 days p.i. mouse was of type a. Those of the 64 days p.i. mouse, of type b. Gr. (for pig; gray zones represent follicles of rows B, lower left, and D. heavy line demarcates representation of follicles of row C). Recordings from affected follicles could be correlated with their reinnervation. In the difference between type a and b, depending on peripheral events, or does it reflect two modes of central adaptation to peripheral injury? Support. Swiss NSF grant 31.158.

33.8.1


The cerebrophen (PC) of the terrestrial slug, Limax maximus, is a region of densely interconnected local olfactory interneurons within which the olfactory interneurons interact with (1) input fibers from the external sensory pathways and (2) intrinsic modulatory fibers containing SCP or FMRFamide and (3) extrinsic modulatory fibers containing serotonin or dopamine.

To search for additional connections between the PC and other areas of the cerebral ganglion, localized injections of HRP were made into the cerebral and synaptic zones of the PC. Fiber tracts to the olfactory nerve were seen, as were isolated single fibers connecting with the metacerebral lobe of the cerebral ganglion.

Material for fine structural analysis was fixed in glutaraldehyde and paraformaldehyde, postfixed in osmium tetroxide and stained in uranyl acetate and lead citrate. The cell mass consists of groups of very closely associated somata surrounded by bundles of axons. Within the synaptic interconnect zone fibers vary widely in size and orientation, containing both clear and electron-dense vesicles.

Dissociated PC neurons in culture are excited by serotonin and dopamine, while SCPF and FMRFamide have little or no direct effect. Responses were assayed by whole-cell loose-patch recordings with pressure pulses of transmitter applied just upstream of the neuron being recorded.

NEURAL PLASTICITY IN ADULT ANIMALS: CEREBRAL CORTEX

33.8.3


Previous [14C]-2-deoxyglucose (2DG) studies revealed an enlarged and diffuse pattern of labeling of C3 representation in contralateral S1 90 days after neonatal somatosensory ablation sparing one vibrissa (C3). In a subsequent study rats kept the spared, enriched C3 vibrissa in contact with the wall of a cylindrical open field but preference dissipated with experience. Since the behavior extinguished in the open-field, this study examined behavioral changes in a more difficult task. On PND 1-3, 11 rats had follicle-ablated unilaterally sparing C3; 8 animals were controls. On PND 60, rats were tested in a darkened cylindrical plexiglass apparatus with a circular runway (5 cm in diameter). Animals were placed against the inner wall of the cylinder 45 cm above the floor. Testing occurred 4 min per day for 5 days. It was predicted that animals with a right spared C3 would keep the vibrissa in contact with the wall of the runway. Clockwise (CW) behavior was scored as correct, while counterclockwise (CCW) left spared C3 rats would travel CW. [14C]-2DG experiments followed behavioral testing. The side of the spared vibrissa affected behavior (p<0.05) which did not extinguish: 9 of 11 spared C3 rats preferred the predicted mode unlike the control animals. The behavioral results correlate with the results of the 2DG tests suggesting that the elevated maze detects somatosensory behavioral changes and functional reorganization.
PLASTICITY IN THE BARREL CORTEX OF ADULT MOUSE: EFFECTS OF PERIPHERAL DEafferENTATION ON THE FUNCTIONAL MAP, A DEoxy-GlUCOSE STUDY.


Institute of Anatomy, University of Lausanne, Rue du Bugin 9, 1005 Lausanne, Switzerland.

In the adult mouse whisker-to-barrel pathway, the deoxyglucose (DG) method has shown that the deafferentation of whiskers evokes an increased responsiveness of the corresponding barrels. In the present study, we used the deoxyglucose method to map the cortical representations of the peripheral sensory fields. The results indicate that the deoxyglucose method is a useful tool for mapping the peripheral sensory fields, and that the changes in the functional map are due to the deafferentation of the peripheral sensory fields.

Although the cholinergic innervation of the cerebral cortex has been reported to play a role in the formation and maintenance of the functional map, the exact role of acetylcholine in this process is still not well understood. The present study was designed to investigate the role of acetylcholine in the formation and maintenance of the functional map using the deoxyglucose method.

In the present study, we used the deoxyglucose method to map the functional map of the cortical representations of the peripheral sensory fields. The results indicate that the deoxyglucose method is a useful tool for mapping the peripheral sensory fields, and that the changes in the functional map are due to the deafferentation of the peripheral sensory fields. The results also suggest that the changes in the functional map are due to the deafferentation of the peripheral sensory fields, and that the changes in the functional map are due to the deafferentation of the peripheral sensory fields.

338.8 MANIPULATION OF CORTICAL CHOLINERGIC INNERVATION ALTERS STIMULUS-EVOKED METABOLIC ACTIVITY IN CAT SOMATOSENSORY CORTICAL AREAS. W. Ma, C.F. Holmes, S. L. Juliano, J.B. Ushui, B. She, B. Johns Hopkins University, Baltimore, MD.

Although the cholinergic innervation of the cerebral cortex has been reported to play a role in the formation and maintenance of the functional map, the exact role of acetylcholine in this process is still not well understood. The present study was designed to investigate the role of acetylcholine in the formation and maintenance of the functional map using the deoxyglucose method.

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338.11 ALTERATIONS IN DENDRITIC EXTENT OF PYRAMIDAL CELLS IN ADULT MOUSE CORTEX: INFLUENCE OF RARE NEUROTRANSMITTERS, R.J. Krier*, K.C. Bartlett and W.H. Sillers, University of Maryland School of Medicine, Baltimore, MD 21201.

In order to establish baseline lifespan parameters of neuronal morphology and assess neuroplasticity in the brains of adult animals, we examined the dendritic structure of pyramidal neurons on the basis of their length and thickness. These results showed that the amount of dendritic material in the 8-month-old mouse was significantly reduced compared to that of the 3-month-old mice: there was a 25% decrease in dendritic material. There was no additional reduction in dendritic extent of pyramidal cells between the 8-month-old and the 13-month-old mice. These findings suggest that in this rodent the transition from early to middle adulthood is associated with an extensive reorganization of dendritic arbor as shown by the reduction of dendritic material. However, in the 8 to 13-month-old range the dendritic field was shown to be stable. (Supported by the National Institute of Neurological and Communicative Disorders and Stroke.)

338.13 ONTOGENY OF NEURONAL DISCHARGE PATTERNS IN THE OCCIPITAL CORTEX OF CHLORAL-HYDRATE ANAESTHETIZED RATS. M.A. Corner and M. Mirmiran*. Netherlands Institute for Brain Research, 1105 AZ Amsterdam.

Male Wistar rats varying in postnatal age from 2 to 12 weeks were studied under chloral hydrate anaesthesia, using glass microelectrodes to record spontaneous firing in single neurons at different depths within the occipital cortex. Computerized quantification of the spike trains revealed a wide variety of complex patterns which differed in their discharge amplitudes and the way in which the POST-SPONTANEOUS potentials indicated at different ages: i) at 1-2 weeks, the spikes were short and of high amplitude, ii) at 2-3 weeks, the spikes were longer and of medium amplitude, iii) at 4-5 weeks, the spikes were longer and of low amplitude, iv) between 5 and 8 weeks, slow (longer than 2-3 min) rebounds occurred in an orderly fashion for different depths within the occipital cortex. Computerized quantification of the spike trains revealed a wide variety of complex patterns which differed in their discharge amplitudes and the way in which the POST-SPONTANEOUS potentials indicated at different ages: i) at 1-2 weeks, the spikes were short and of high amplitude, ii) at 2-3 weeks, the spikes were longer and of medium amplitude, iii) at 4-5 weeks, the spikes were longer and of low amplitude, iv) between 5 and 8 weeks, slow (longer than 2-3 min) rebounds occurred in an orderly fashion for different depths within the occipital cortex. Computerized quantification of the spike trains revealed a wide variety of complex patterns which differed in their discharge amplitudes and the way in which the POST-SPONTANEOUS potentials indicated at different ages: i) at 1-2 weeks, the spikes were short and of high amplitude, ii) at 2-3 weeks, the spikes were longer and of medium amplitude, iii) at 4-5 weeks, the spikes were longer and of low amplitude, iv) between 5 and 8 weeks, slow (longer than 2-3 min) rebounds occurred in an orderly fashion for different depths within the occipital cortex.

338.12 FLUORESCENT STAINING OF THE CEREBRAL CORTEX IN LIVING MICE. A.J. LaMattina, S.L. Fiume, D. Purves, Dept. of Pathology & Neuroscience, Washington University School of Medicine, St. Louis, Missouri 63110

We have evaluated the ability of several vital dyes to stain the superficial layers of the cerebral cortex of mouse brain slices (0.25 mm thick) in vivo. The dyes used were: calcein AM (20, 5-µM), propidium iodide (100 µM), 4',6-diamidino-2-phenyl-indole (1 µM), and 4',6-diamidino-2-phenyl-indole dihydrochloride (50 µM). The dye concentrations were chosen to optimally stain the superficial layers of the cerebral cortex and to allow visualization of neuronal morphology. The dyes were applied to the cerebral cortex of living mice and allowed to incubate for 15 min before being washed with phosphate-buffered saline. Photomicrographs were taken using a Zeiss plan-apochromat 40X objective and digital image capture software. The fluorescent dyes were found to stain the superficial layers of the cerebral cortex of living mice. The calcein AM and propidium iodide stains allowed visualization of neuronal morphology. The 4',6-diamidino-2-phenyl-indole dihydrochloride (50 µM) stain allowed visualization of neuronal morphology. The 4',6-diamidino-2-phenyl-indole dihydrochloride (50 µM) stain allowed visualization of neuronal morphology. The 4',6-diamidino-2-phenyl-indole dihydrochloride (50 µM) stain allowed visualization of neuronal morphology.
5-HT, RECEPTORS IN RAT PREFRONTAL CORTEX. R.C. Arias-Gra* and P. Andrade* (Spon: J.G. Goldfarb). Dept. of Pharmacology, St. Louis Univ. School of Med., St. Louis, MO 63104.

While the pharmacology, distribution and biochemistry of central 5-HT receptors have been extensively investigated, little is still known about the physiological responses they mediate. Therefore we have used intracellular recordings in vitro rat cortical slices to examine the effects of 5-HT on the prefrontal cortex, an area highly enriched in these receptors.

Bath administration of serotonin (1 AM - 30 µM) elicited three distinct spikerelated and ketanserin sensitive responses. These included a small, subthreshold depolarization associated with a conductance decrease, a reduction in the slow afterhyperpolarization which follows a burst of spikes, and a marked decrease in spike frequency accommodation. In addition, when 5-HT7 and 5-HT3 receptors coexisted on the same cell, activation of the 5-HT7 receptors reduced or blocked the ability of the 5-HT3 receptors to hyperpolarize these cells.

Thus, activation of 5-HT7 receptors in this region elicits a set of distinct actions which interact to produce an increase in excitability to incoming stimuli while blocking the inhibitory actions of 5-HT.

Supported by the Pharmaceutical Manufacturers Association Foundation.

EFFECTS OF KETANSERIN PRETREATMENT ON MMD-INDUCED SEROTONIN (5-HT) DEPLETION IN THE RABBIT. J.P. Walsh and H.Y. Nemer, Department of Psychiatry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

Previous studies conducted in our laboratory have established that 3,4-methylenedioxymethamphetamine (MDMA) stimulates the secretion of corticotropin in a dose- and time-dependent manner, and that ketanserin pretreatment attenuates this effect of MDMA. MDMA has been reported to elevate brain concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in a dosicentric manner. The present study was undertaken to determine the effect of ketanserin/pretreatment on acute (3 hr) and chronic (7 day) MMD-induced 5-HT and 5-HIAA depletions.

A single injection of MDMA (30 mg/kg, sc.) significantly reduced the concentration of 5-HT and 5-HIAA in the following brain areas: Frontal cortex > Hippocampus > Hypothalamus at 3 hr and 7 days. Treatment of rats with ketanserin (3 mg/kg, ip) 1 hr prior to MDMA administration significantly attenuated MDMA-induced depletion of 5-HT and 5-HIAA at both time points. Ketanserin prevented MDMA-induced depletion most significantly in the striatum followed by the hippocampus and frontal cortex. MDMA-induced depletion of 5-HT and 5-HIAA in the hypothalamus was unaffected by ketanserin. These data suggest that reducing the acute MDMA-induced depletion of 5-HT and 5-HIAA by ketanserin pretreatment attenuates the long-term depletion. We propose that overstimulation of 5-HT, receptor mediates, in part, the acute and long-term depletions of 5-HT following a single administration of MDMA.

5-HT, RECEPTORS IN THE RABBIT RETINA? W.D. Brunton and N.H. Blasius, Dept. of Biology, Boston College Chestnut Hill, MA 02167 and Dept. of Cell Biology, Washington Univ. St. Louis MO 63110

We have suggested previously that both 5-HT, & 5-HT, receptors play a role in signal processing in the rabbit retina (JNS 7:1061-1065). In preliminary experiments we have employed MDI 72222 and ICS 205-930, serotonin antagonists selective for 5-HT, receptors in a superfused isolated eye cup preparation to test if 5-HT, receptors affect the visual responses of the ganglion cell, the output neuron of the retina.

These drugs have reversible effects on the visual responses of both ON and OFF center brisk cells. For all cells tested, the ON-excitation was reduced and in some cells the OFF-excitation was increased. The spontaneous activity of all classes of cells was also decreased. These effects were reversed in 10 to 20 minutes after the drugs were washed out of the perfusion bath.

The results obtained with these agents are similar in all respects to those obtained with agents selective for 5-HT, receptors. These results may imply either that 5-HT, receptors are present in the mammalian retina or that the reputed 5-HT, agents have actions at central 5-HT, receptors. Further experimentation will be necessary to confirm a functional role for 5-HT, receptors in the retina.

(Sponsored by FY 06776 WJB and FY 00053 NDB)
339.9 SEROTONIN-3 RECEPTOR MEDIATED SPINAL ANTI-

We recently reported that the potent and selective 5-HT3 receptor antagonists GR 38032F (GR) and MDL 72222 inhibit specific 3H-Serotonin binding to purified rat dorsal spinal cord synaptosomal membranes (Eur. J. Pharmac., submitted). Intrathecal (10 µg) administration of GR is known to produce significant antinociception as measured by increased tail-flick latency (TFL). We now report that the selective 5-HT3 receptor agonist 2-methyl-5-HT mimics the antinociceptive action of 5HT. Additionally, the selective 5-HT receptor antagonist ICS 205-930 (ICS) inhibits 5HT-induced antinociception.

GR 38032F (1,2,3,9-tetrahydro-9-methyl-3-[2-
methylimidazol-1-yl]-6-carboxy-1,4-benzodiazepine-1,4-dione, hydrochloride) is a potent and selective 5-HT3 receptor antagonist (Brittain et al., Br. J. Pharmac. 90: 87P, 1987) that has been shown to decrease mesolimbic DA neurotransmission (Ahlberg et al., Br. J. Pharmac. 90: 87P, 1987). We have also found that the selective 5-HT3 receptor antagonist GR 38032F (1 µg/iv) produced no effect on the baseline firing rate of DA neurons in the nucleus accumbens of chloral hydrate-anesthetized rats. IV administration of GR 38032F in cumulative doses up to 1 mg/kg had no effect on the baseline firing rate of DA neurons. Pretreatment with GR 38032F (1 mg/kg iv) did not alter the inhibitory effects of d-amphetamine but did attenuate the excitatory effects of 5-hydroxytryptamine (5-HT) on A10 DA neurons. These preliminary electrophysiological results support the contention that GR 38032F may selectively affect DA hyperactivity without altering basal activity.

339.10 5-HYDROXYTRYPTAMINE INFLUENCES RELEASE OF DOPAMINE AND ITS METABOLITES IN THE RAT STRIATUM. N. M. Deutch and M. J. Melmed. Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, New York, NY 10029.

Serotoninergic fibers originating from the dorsal raphe nucleus project into the striatum but the function of 5-hydroxytryptamine (5-HT) in striatum is still unclear since the neurotransmitter has been reported to have both excitatory and inhibitory effects (Chesebro and Gage, J. Neurochem. 54: 547, 1990). The role of 5-HT in the release of endogenous dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from superfused striatal slices was assessed. Eight week old, male Sprague-Dawley rats were decapitated, and striatal slices (400 µm thick) were superfused with artificial cerebrospinal fluid (acSF) containing nafamistine (10 µM) to block DA reuptake. Measurements were made by high performance liquid chromatography with electrochemical detection. Spontaneous release of DA from the slices averaged 1.9 pmol/mg protein/3 min. The release of DOPAC and HVA averaged 3.7 and 1.1 pmol/mg protein/3 min, respectively. Superfusion with 5-HT (10µM) resulted in a 3-fold elevation of DA release with no significant changes in the release of DOPAC or HVA. The 5-HT agonists 5-carboxamidotryptamine (1 µM) and 2,5-dimethoxyphenylisopropylamine (10 µM) were ineffective in increasing the spontaneous release of DA. DOPAC or HVA. The 5-HT receptors that have been described in the peripheral nervous system where they appear to mediate the excitatory effects of 5-HT. These data suggest that the 5-HT3 receptor is present in brain as well. Supported by a grant (DA 08751) from the National Institute on Drug Abuse.

339.11 EXAMINATION OF THE EFFECTS OF THE 5-HT3 RECEPTOR ANTAGONIST MDL-72222 ON PUNISHED RESPONSES IN PIGEONS. S. L. Ablera and J. K. Bartes. Department of Psychiatry, Uniformed Services University of the Health Sciences and the Naval Medical Research Institute, Bethesda, MD 20814.

MDL-72222, a 5-HT3 receptor antagonist, was examined for potential anxiolytic activity using a behavioral assay that, in general, has correlated well with clinical efficacy in humans. In pigeons, the punished responses of some pigeons within a narrow dose range of 0.1-1.0 µg/kg, i.m. administered immediately before the session produced slight increases in punished responding of 0.1-0.3 mg/kg. Unpunished response rates were unaffected across most of the dose response curve. Overall, the effects of MDL-72222 on punished responding were substantially weaker than comparable to buspirone or chloridiazepoxide which have robust effects under this procedure and show potent anxiolytic activity in humans. Supported by PHS Grant DA-02873.


Based on reports of possible antipsychotic utility of serotonin-3 (5HT3) antagonists, we examined the effects of the reported 5HT3 antagonists GR 38032F (GR), MDL 72222 (MDL) and ICS 205930 (ICS) in locomotor activity tests in rodents. In mice, MDL reduced spontaneous locomotion with an ED50 of 5 mg/kg ip and did not cause ataxia at active doses, a profile seen with dopamine antagonists. GR reduced locomotion at higher doses (ED50 30 mg/kg ip) in mice and also did not cause ataxia. GR did not reduce locomotor activity in rats at doses of 0.001-3 mg/kg ip; higher doses were lethal. In rats, MDL reduced locomotor activity after oral dosing with an ED50 of 8 mg/kg; GR and ICS were inactive at doses up to 10 and 100 mg/kg, respectively. GR failed to reduce locomotion in rats after SC or IP dosing with up to 10 mg/kg. Neither acute nor BID dosing with GR for 3 successive days reduced d-amphetamine-induced hyperactivity in mice. In contrast, both MDL and ICS antagonized amphetamine stimulation in mice. These results indicate that while the profiles of MDL 72222 and ICS 205930 in these tests share common features with those of dopamine antagonists, this activity is not seen with the other 5-HT3 antagonist, GR 38032F.
339.15

NEUROENDOCRINE RESPONSES DEMONSTRATING A FUNCTIONAL
REGULATION OF THE 5-HT RECEPTOR AFTER CORTICOSTERONE IN

The present studies were designed to determine whether the 5-HT receptors which increase renin and corticosterone (CORT) secretion are pre- or post-synaptic. Rats treated with nomifensine (15 mg/kg, i.p.) to protect catecholamine terminals, were injected bilaterally with 5,7-DHT (75 µg/10 µl/site i.c.v.) to destroy 5-HT nerve terminals. Two weeks later, the rats received an injection of the 5-HT agonist RU24969 (0.2, 1.0, or 5.0 mg/kg, i.p.) or saline. At sacrifice, in 5,7-DHT treated rats, there was a shift to the left in the dose-response curve for the effect of RU24969 on CORT release. The minimal dose which significantly increased CORT, decreased from 5.0 mg/kg to 1.0 mg/kg. The dose which produced a significant increase in plasma renin activity/ concentration also decreased from 0.2 mg/kg in the vehicle group to 0.02 mg/kg in 5,7-DHT treated rats. In conclusion, the neuroendocrine response to the 5-HT agonist RU24969 was not blocked after the destruction of 5-HT nerve terminals, suggesting that the 5-HT receptors which mediate this response are located post-synaptically.

The shift in the dose response curves suggests functional desensitization supersensitivity of the 5-HT receptors.

339.17


Children with congenital hyperammonemia (HA) develop cerebellar ataxia by 15 days of age, which is associated with neurologic and metabolic derangements including anemia and changes in activity and sleep. Osmotic minipumps were implanted i.p. to deliver urea,4.5 U1290g/d, into halothane anesthetized rats and produce HA. Behavioral alterations included anemia and increased activity changes; a few rats died. After HA, plasma ammonia (n=5 each) prior to sacrifice at 12, 24 and 48 h. after implant were 1600, 2960, 4900 µM vs sham animals levels of 77-130. Some non-polar and large hydrophobic amino acids were increased in cortex. There were 2-4 fold increases in levels of tryptophan, glutamine and 5-HIAA in cortex, with no significant changes in quinolinic acid, the other major pathway for tryptophan metabolism nor in other biogenic amines. We conclude that urea infusion is an apt model of congenital hyperammonemia and that there is an increase in serotonin metabolism that may relate to the neurobehavioral abnormalities in this disorder.

339.19


Buspirone has been reported to have broad spectrumed antiemetic efficacy in cats. Its site of action required verification by testing 8-OH-DPAT against the same emetic stimulus. 5-HT3 antagonists have been reported to prevent radiation-induced emesis in ferrets. The antiemetic effects of these drugs was tested against three emetic stimuli in cats and compared with 5-HT3 agonists. The emetic stimuli were: 1) motion in a devise similar to a Ferris wheel, 2) 0.66 mg/kg xylazine SC and 3) 7.5 µg/kg cisplatin i.v. Cisplatin induced sickness with an EDSO of 0.011 mg/kg and xylazine-induced emesis with an EDSO of 0.056 mg/kg. The dose of 0.64 mg/kg xylazine completely abolished xylazine-induced emesis. IC50 205 930 (1 and 0.1 mg/kg) and zucaprid (0.01-10 mg/kg) did not prevent motion sickness and IC50 930 and MEL 72222 did not prevent xylazine-induced emesis. 5-HT3 antagonists had greater efficacy in preventing cisplatin-induced emesis but stimulation of 5-HT3 receptors had efficacy against several stimuli. The two classes of drugs appear to act through different mechanisms.

339.16


An age-related decline in presynaptic 5-HT function has been identified from 5-HT receptor binding sites in brain tissue from aged female rats. Female Fischer 344 rats (Young vs Old) were sacrificed (7 days), vehicle implanted (sesame oil) for 848 hr and sacrificed. Brain tissue was removed, dissected and stored at -80°C until assayed. Radioligand binding studies were carried out using [3H]5-HT (0.05-32 nm), with tissue diluted 1:25 in Tris-SC buffer; nonspecific binding was determined using 10 μM 5-HT. Scatchard analysis were performed using LIGAND (Snelveez; Coef. C> 0.90) and protein values determined by Bio-Rad assay. No alterations in receptor number (1max) were detected in Young vs Old prefrontal cortex (PFC) (168 vs 145 pmol/mg prot.) and DRN (166 vs 160 pmol/mg prot.). In contrast, receptor affinity apparently decreased with age (Young vs Old) in the PFC (Kd 10.9 vs 15.4 µM) but not the DRN (Kd 16.8 vs 17.2 µM). These data suggest that age related changes in 5-HT PPC but not DRN receptor binding sites occur in the female rat. Further investigations are evaluating age-induced changes in 5-HT receptor subtypes. Supported by AG 06017.

339.20


Activation of MEL receptor sites modulates the activity of norepinephrine (NE) neurons innervating the hypothalamus of C3H/HeJ mice (Fang and Dubocovich, PASEB J. 5: A182, 1988). The role of endogenous MEL activity was assessed by blocking MEL receptor sites with the antagonist luzindole (LUZ, 30 mg/kg, i.p., 30 min). The levels of NE (mg/kg) in the hypothalamus of C3H/HeJ mice were determined following inhibition of NE turnover with α-CH3-p-tyrosine (α-Mpt, 300 mg/kg, i.p., 2 h) by HPLC with EC detection. At noon, when the levels of endogenous MEL are low, LUZ did not affect the current levels of NE. The levels of NE were significantly decreased with α-Mpt and LUZ (0.3 ≤ Z > 0.03 Z < 3.0 with different controls). Depending on administration route, however, Z produced side effects of retching/emesis and defecation in the ferret (Kung et al., PASEB J 2: A358, 1988). We are currently evaluating MEL for similar effects.
340.1 DOPAMINE AGONISTS MAINTAIN MAGNESIUM INDUCED CONDITIONED PLACE PREFERENCE. J.K.M. Kantak, Lab. of Behav. Neurosci., Dept. of Psychol., Boston University, Boston, MA 02215.

Data suggests that the mesolimbic dopamine system is involved in mediating sexual arousal (Baum & Starr, Pharm. Biochem. & Behav., 13, 175, 1980; Cagnoli et al., Brain Res., 1112, 1976). Dopamine is known to increase the activity of dopaminergic cells (Matthews & German, Neurosci., 11:617, 1984; Gysling & Wang, Brain Res., 227,119, 1983), and when applied to the region of mesolimbic DA cell bodies, the VTA, increases locomotor activity (Joyce & Iversen, Neurosci., 15, 207, 1991; Vezina & Stewart, Pharm. Biochem. & Behav., 22, 975, 1989), and indicates that Hamilton & Bozarth, Behav., 13, 412, 1987). The opioid peptide, dynorphin, applied to the VTA also increases feeding (Hamilton & Bozarth, 1977).

340.2 THE EFFECTS OF INTRA-VTA INFUSIONS OF MORPHINE AND DOPAMINE ON SEXUAL BEHAVIOR. J. L. et al., Lab. of Behav. Neurosci., Dept. of Psychiatry, Yale University, New Haven, CT 06510.


A comparison of anabolic steroid administration with psychological assessment of P-1 receptor supersensitivity demonstrated the presence of biochemical markers of psychosis during steroid administration. Twenty-five male page subjects received 6 weekly IM injections of 100 or 300 mg testosterone enanthate (Te), 100 or 300 mg nandrolone deconate (Nan). Two blood samples were drawn on different days before drug administration, and two were drawn during the 6th wk after the final drug dose. Serum levels of HVA and 5-hydroxyindolacetic acid (5-HIAA) were determined by HPLC with dual electrode detection and separated with two mobile phases to confirm the purity of the eluted compounds. Increases in plasma HVA were found in both Nan groups but not in the Te subjects. Plasma HVA is an indicator of tissue dopamine turnover.


We have recently shown in rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA) for 14 days, that the expressivity of the dopamine system is dependent on the previous "priming" with a DA receptor agonist. In this study we examined the temporal and the dose-dependency characteristics of this phenomenon. Male Sprague-Dawley rats lesioned with 6-OHDA were used throughout all the experiments. Administration of the 0-receptor agonist SKF38393 (2mg/kg) failed to elicit any contralateral or central lateral turning (c.) in naive rats lesioned from 14 days, but produced an intense contralateral turning in naive rats lesioned from 90 days. A single administration of the 01-receptor agonist SKF38393 (0.1mg/kg, 3 days before) which produced c. by itself, made SKF38393 very active in inducing c. in 14 days lesioned rats. The "priming" induced by apomorphine develops with time, in fact administration of SKF38393 three hours after apomorphine, failed to elicit c. Like apomorphine, also the apomorphine receptor agonist LY171555 and SKF38393 itself induced "priming" in 6-OHDA lesioned rats from 14 days. This "priming" however was strictly dependent from the dose of the drug used. Moreover "priming" with any of the mentioned drugs was ineffective in rats lesioned from 7 days. The results indicate that the expression of "priming" depends from the time of "priming" and from the dose of the drug used for "priming".

340.5 LESIONS OF THE SUBSTANIA INNOMINATA UNMASK AN INHIBITORY EFFECT OF APOMORPHINE ON ACOUSTIC STARTLE. C.B. Sances, J.M. Hitchen, B. Ross, M.D. Mirando and A. M. Davis. Dept of Psychiatry, Yale University, Rippolus Research Fac., Conn. Mental Health Ctr, New Haven, CT, 06508.

Dopamine receptor subtype agonists have opposite effects on the acoustic startle reflex. D1 agonists increase startle whereas D2 agonists depress it. The net effect of the mixed agonist is an enhancement of startle. However, this effect can be reversed into inhibition by pretreatment with the D1 antagonist SCH 23390. The present study sought to determine whether various dopaminergic agonist antagonists are involved in apomorphine's effect on startle.

340.6 DIFFERENTIAL EFFECTS OF SELECTIVE DOPAMINE AGONISTS AND ANTAGONISTS ON STARTLE ELICITED ELECTRICALLY FROM THE BRAINSTEM. R.L. Matsa & M. Davis (SPON: W.P. Jordan). Dept of Psychiatry, Yale University, New Haven, CT 06508.

The mixed D1, D2 dopamine agonist, apomorphine, is known to increase the amplitude of the acoustic startle. Pretreatment with the selective D1 antagonist, Sch 23390, reverses the usual excitatory effect of apomorphine into an inhibitory one. Pretreatment with the D2 antagonist, sulpiride, augments the excitatory effect of apomorphine. These data suggest that activation of D1 dopamine receptors increases startle whereas activation of D2 dopamine receptors decreases startle. Acoustic startle is mediated by a neural circuit consisting of the ventral cochlear nucleus (VCN), the ventral intermediate nucleus (VIN), the ventral basomedial nucleus (VBM), the lateral pontine nuclei (LPN) and the spinal cord. By elicting startle electrically at different points along this pathway the present study sought to determine where D1 and D2 receptor activation ultimately alters neural transmission so as to have opposite behavioral effects.

Previous studies have shown that apomorphine increases startle elicited by an acoustic stimulus but decreases startle elicited by electrical stimulation of the VCN. In the present study the D2 agonist, Lilly 171555, was found to be ineffective on startle. The excitatory effect of apomorphine was mediated by its D1 activity and impinges upon the startle pathway at or before the VCN while apomorphine inhibitory effect is mediated by its D2 activity and impinges upon the startle pathway supraspinally but downstream from the VCN.
340.7
EFFECTS OF ALPHA-METHYL-PARA-TYROSINE ON THE MAINTENANCE OF CONDITIONED PLACE PREFERENCE. R. Birose* and N.H. White, Department of Psychology, McGill University, Montreal, Canada.

When dopaminergic function in nucleus accumbens is disrupted on the training days in the conditioned place preference (CPP) paradigm, CPPs for amphetamine or food are not observed, suggesting that this neurochemical mediates some aspect of primary reward. The purpose of the present study was to determine if normal dopamine function is necessary on the test day to observe a preference previously conditioned with amphetamine. Rats were given 12 training days in a CPP apparatus, including 6 pairings of 2 mg/kg d-amphetamine with one part of the apparatus and 6 pairings of saline with the other part in a counterbalanced manner. Bilateral injections of 80 μg of α-MPT into nucleus accumbens prior to testing had no effect on the CPP observed, compared to animals receiving saline or no injections. In a control experiment, bilateral injections of the same dose of α-MPT into nucleus accumbens significantly suppressed the locomotor activity produced by 2 mg/kg of d-amphetamine without causing motor impairment, suggesting that this treatment blocks dopamine release. Although there is evidence suggesting that dopamine in nucleus accumbens mediates some aspect of primary reward, the present result suggests that the memory of the primary reward, which is expressed as a place preference on the test day, is not mediated by dopamine release.

340.8
DOPAMINE D1 AND D2 RECEPTORS AND LOMOCOMOTIVE ACTIVITY ELICITED FROM THE NUCLEUS ACCUMBENS OF RATS. M.J. molds and D.M. Jackson. Pharmacology Department, Sydney University, N.S.W. 2006, AUSTRALIA.

While it is well documented that the injection of DA agonists into the accumbens (Acb) of rats produces locomotor activation, it is less clear which DA receptor subtype is important and whether there is any interaction between them. Early studies suggested that D1 receptors played a role, with clonidine, dipyridamole-cyclic-AMP and SKF38393 (SKF) producing excitation after direct injection. In the present study, SKF (10 μg) plus ketanserin (0.1 mg/kg, ip) and spiperone (0.1 mg/kg), but not by ketanserin. The D1 agonist, CY208-243 (CY) was only slightly active (up to 8 μg), and the activity lasted for 2 h. Quipazine (0.3 to 3.6 μg) increased activity for about 2 h. DA depletion blocked the stimulant effects of SKF, CY and quipazine. However, stimulation was produced in these DA-depleted rats when SKF quin plus quin plus quin was injected into the Acb. The activity consisted of coordinated movements and rearing and increased with higher doses of stereotyped. The stimulation lasted for about 5 h (SKF/quin) or 2 h (CV/quin). In rats with their DA stores intact, SKF (or CY) plus quin had an anesthetic effect on stimulating activity. The data clearly show that both D1 and D2 receptors play an important role in mediating stimulation elicited by DA agonists in the Acb. SUPPORTED BY THE NH & MRC.

340.9
EFFECTS OF STIMULATION AND BLOCKADE OF DOPAMINE RECEPTORS ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. B.L. Barret* and J.B. Appel, Behavioral Pharmacology Laboratory Department of Psychology University of South Carolina, Columbia, SC 29208 USA.

The involvement of dopamine (DA) receptor subtypes in the behavioral (subjective) effects of cocaine was studied in rats which were trained to discriminate 10 μg/kg of saline from an equal dose of cocaine. Several DA antagonist treatments significantly reduced the discriminative stimulus effects of cocaine. SKF 38393 (5.0 - 15.0 μg/kg), however, significant amounts of drug-appropriate responding did occur following relatively high doses of d-amphetamine (5.0 μg/kg) and SKF 38393 (0.0625 - 0.25 mg/kg). In combination treatments, the D1 antagonist SCH 23390 (0.065 - 0.5 μg/kg) as well as the D2 antagonist spiperone (0.25 - 0.5 μg/kg) antagonized or attenuated but did not completely block the cocaine cue. These data suggest that DA neuronal systems probably play a role in the in vivo effects of cocaine, but that the stimulus properties of this compound involve mechanisms that are more complex than direct activation of D1 or D2 receptors (e.g. stimulation of both D2 and D1 receptors).

340.10
DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE AND PROCRAINE. B. B. Rush, D. E. Perry and M. A. Charney. Psychiatry, Univ. of Texas School of Medicine, Houston, TX 77030.

The abuse potential of cocaine and other psychomotor stimulants has been attributed to dopaminergic activity as has the discriminative stimulus that results from cocaine administration. Other drugs have been shown to modulate the local anesthetics including procaine, have stimulus properties with similarity to those of cocaine. There is, however, little evidence of dopamine agonist activity by procaine. The work here compared the stimulus properties of cocaine and procaine in naive rats trained to discriminate one of these compounds from saline in a two lever, food reinforced operant procedure. The intent was to shed light on the apparent inconsistency concerning dopamine mediation of the stimuli. The results do not suggest that while cocaine- and procaine-induced stimuli are, indeed, somewhat similar, only other psychomotor stimulants generalized completely to cocaine (and not to procaine) and haloperidol more effectively blocked cocaine than procaine recognition.

340.11

Little is known about the genetic determinants influencing susceptibility to the behavioral actions of cocaine. We have utilized inbred mice as a model system to assess the role of genotype in drug-seeking behavior (DSB). Conditioned place preference (CPP) was used as a behavioral marker for cocaine-induced DSB. Conditioning to environmental cues was carried in a chamber consisting of two compartments, one black with a wire mesh floor and cedar smell and one white with a wood chip floor and pine smell. Mice (n=10 for each treatment) were injected with vehicle or cocaine and placed in the conditioning chamber for 30 minutes. CPP was assessed 24 hours following 1 to 3 daily conditioning trials. Two strains of mice, C7BL/6J and BALB/cByJ, showed significant (p <0.003, dosage trial, dependent variable) stereospecific induction of CPP to the innately less preferred compartment. In contrast, the motor activity-stimulating effects of cocaine differed markedly in the two mouse strains. C7BL/6J showed the expected dosage-dependent decrease in motor activity induced by 18 mg/kg ip. Cocaine failed to induce motor activity stimulation in BALB/cByJ at any dose. These data indicate that genotype can influence the inherent behavioral responsiveness of an animal to cocaine. Relative susceptibility to its motor-activity effects does not predict inherent susceptibility to its reinforcing properties.

340.12

The effects of caffeine and caffeine-phenylalanine combinations upon the discriminative and rate-altering effects of cocaine were examined in rats. Twelve male Sprague-Dawley rats were trained in a two-choice, food reinforced, drug discrimination task with 10 mg/kg cocaine and saline as discriminative stimulus. Stimulus generalization tests with cocaine resulted in a dose-related decrease in cocaine-appropriate responding and variable decreases in response rates. Caffeine also engendered a dose-related increase in cocaine-appropriate responding (but only partial generalization at the highest dose tested) and a biphasic dose-effect curve for response rate. Caffeine potentiated the discriminative stimulus properties of cocaine with a pharmacographic analysis characterizing the interaction as simple additivity. Caffeine's effects upon the rate-reducing effects of cocaine resulted in a biphasic interaction pattern. Rates were also tested with a wide range of cocaine doses and several dose combinations of caffeine, epinephrine, and phenylpropanolamine. The CEP combinations supported by Okla. Dept. Commerce 1686 and NIDA DA04444.
341.3 DEPLETION OF SPINAL CORD NORADRENALINE ALTERS ANTINOCICEPTIVE EFFECTS INDUCED BY IMPLANTED NUCLEUS RAPHE MAGNUS STIMULATION. Arab, S. and Freedfill, J.K. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

We examined the capacity of spinal cord noradrenaline (NA) depletion to attenuate the antinociception produced by the interaction between intrathecal (IT) α2-adrenergic hexamethonium (HEX) and release of endogenous NA produced by nucleus raphe magnus (NRM) stimulation.

Animals received an IT injection of either vehicle or IT HEX. Twenty one days after surgery baseline tail flick latencies (TFLs) were determined and a stimulus response curve was generated for electrical stimulation of the NRM. Animals were then given an IT injection of either vehicle or NA-depleted NRM stimulation. NA stimulation produced significant increases in TFLs both before and after NRM injection in animals pretreated with vehicle. In animals injected with NA, NRM stimulation failed to increase TFLs.

These data suggest that endogenous NA, released by NRM stimulation, interacts synergistically with NA to produce antinociception.

341.4 MLD 26,764, A NON-NARCOTIC ANALGESIC WITH α2-ADRENERGIC PROPERTIES. F.P. Miller, D.L. Braun, B.J. Ketteler and A.A. Carr, Merrell Dow Research Institute, Cincinnati, OH 45215.

Analgesic activity of MLD 26,764 ([1-piperidine-ethanol]-α,4-(fluorophenyl)-4-{[4-(fluorophenyl)]hydroxy-methyl}-] was assessed against acetic acid-induced writhing in male rats. Doses varying from 10-100 mg/kg administered 30 min after sc administration, were 1.85 and 4.73 mg/kg, respectively. This activity was present for at least 4 hrs in mice (ED50 values of 8.73 mg/kg sc and 27.6 mg/kg po). MLD 26,764 also produced analgesic activity after intraventricular (ED50 19.3 μg/mouse) or intrathecal (ED50 14.7 μg/mouse) administration. In mice, MLD 26,764 increased response latency in the tail immersion test, but was ineffective in altering response latency in the hot plate test. The analgesic activity of MLD 26,764 was completely antagonized by systemic administration of the selective α2-antagonist, idaroxan(1), whereas benztrafine, an α2-antagonist that does not cross the blood-brain barrier, and the opiate antagonist, naloxone, were ineffective. Colonic motility in mice, known to be altered by α2-agonists, was inhibited by MLD 26,764; this inhibition was reversed by 1. These results indicate that MLD 26,764 is a centrally acting, non-narcotic analgesic with a mechanism of action dependent primarily on an agonist-like effect on α2-adrenergic receptors.

341.5 INHIBITION OF NSAID-INDUCED ANTINOCICEPTION IN MICE BY ALPHA-2 ADRENERGIC RECEPTOR BLOCKERS. J.A. Balsam*, N. Reglin*, D. Koos* and D. Luttinger. Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Central alpha-2 adrenergic blockade has been reported to attenuate antinociception produced by morphine (e.g. Camarata and Yako, Brain Res. 1985). We now report that the antinociceptive effects in the mouse acetylcholine-induced writhing test of two non-steroidal antiinflammatory analgesics (NSAIDs), naproxen and zomepirac, are also attenuated by alpha-2 adrenergic receptor antagonists.

Male Swiss-Webster mice were treated with receptor antagonists (α) 2 hours prior to administration of NSAID (1x) then challenged with acetylcholine (ACH: 3.2 mg/kg Ip) or phenyl-pseudoquinoine (PFQ: 1 mg/kg Ip). S.C. administration of zomepirac (3 mg/kg) or ibuprofen (IBX:1884; 1 mg/kg) shifted the naproxen dose-response curve to the right. Antinociceptive effects of another NSAID, zomepirac, were also inhibited by yohimbine pretreatment. The writhing response to ACh was not affected by yohimbine pretreatment, suggesting that nociceptive thresholds to ACh were not altered. Yohimbine (3 mg/kg, s.c.) did not alter the antinociceptive effects of naproxen in the PFQ writhing test.

These data suggest that alpha-2 adrenoceptor agonists can modify antinociception induced by NSAIDs. However, these effects may be dependent on the nociceptive stimulus used. It is unclear whether this is due to differences in intensity of nociceptive stimulus or to mechanisms of inducing noiceception.

341.6 VISCERAL NOCICEPTION: INHIBITION BY SPINAL α-ADRENOCEPTORS. B.H. Pennebaker and G.F. Gehalt, Department of Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

The antinociceptive effects of the intrathecal administration of the α-adreceptor agonists clonidine, ST-91, and tizanidine, the nonselective α-agonist isoprenaline, and the α-agonist isoproterenol were examined in awake, unanesthetized rats. Colonic distension, the noxious visceral stimulus employed, elicited a vigorous pressor response and contraction of abdominal and hindlimb musculature (a visceral nociceptive). Chronic intrathecal and arterial catheters were implanted. At the time of experimentation a dissectible, latex balloon was inserted nonsurgically via the anus into the descending colon and rectum, and distensions were given 3 minutes apart. All drugs were administered in equal volumes, 7.5 μl, followed by a 7.5 μl flush of saline. Cumulative doses were given at 12 minute intervals. The α-adrenoceptor agonists produced a dose-dependent attenuation of the pressor and visceral nociceptor responses. Pretreatment with the α-adrenoceptor antagonist yohimbine antagonized the effects produced by clonidine, ST-91, and tizanidine, whereas the effects produced by norepinephrine were not significantly altered. The α-adrenoceptor agonist isoproterenol did not attenuate the pressor or visceral nociceptor response to colorectal distension. These results demonstrate that spinal α-adrenoceptors, and not β-adrenoceptors, mediate antinociception to noxious visceral stimulation.

The present studies identify a new high affinity 3H-5-HT binding site in spinal cord which has a unique pharmacologic profile not observed in frontal cortex.

The density studies employed 3H-5-HT binding for 3H-5-HT binding in rat frontal cortex and spinal cord was determined in saturation (3H-5-HT, 3H-8-OH-DPAT, 3H-meselergine) and competition studies. In contrast, competition studies (3H-5-HT) employing selective 1A masks (8-OH-DPAT, buspinone), 1B mask (RU24969) and 1C mask (mesulergine) determined that 30% of 3H-5-HT binding sites were of the 1A subtype, 4% of 1B and 56% of 1C subtypes, apparently accounting for all cortical 5-HT receptors. Competition studies in cortex confirmed these estimates. In spinal cord, the same experimental techniques identified that 25% of 3H-5-HT binding sites were of the 1A subtype and 33% were 1B receptors. Subsequent competition studies indicated that the remaining 42% of this high affinity 3H-5-HT binding in spinal cord (Ki=6±1 nM) was not to 5-HTIC, 5-HT2 or 5-HT3 receptors. The exact nature of this high affinity 3H-5-HT binding site is in progress.

LAMINAR DISTRIBUTION OF RAPHESPINAL FIBERS IN THE RAT LUMBAR DORSAL HORN DEMONSTRATING SEROTONIN-LIKE IMMUNOREACTIVITY. E.K. Jones and A.R. Light. Dept. of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599.

The nucleus raphe magnus (NRM) has been implicated in the centrifugal modulation of spinal nociceptive transmission. Serotonergic raphe spinal projections have been demonstrated using the retrograde tracer horseradish peroxidase in conjunction with immunohistochemistry, however, the laminar distribution of serotonin-containing raphe spinal fibers and terminals within the dorsal horn has not been examined. The purpose of this study was to address this issue by using the anterogradely transported lectin, Phaseolus vulgaris-leucoagglutinin (PHA-L) coupled with immunohistochemistry. Male Sprague-Dawley rats were anesthetized with an IM injection of ketamine/xylazine. Microinjections of PHA-L (10%, 0.2 µl) were made into the ventromedial medulla and the rats were allowed to recover. After 4 weeks, they were perfused transcardially and the spinal cord tissue was removed and reacted with PHA-L antibody tagged with Texas Red and serotonin antibody tagged with fluorescein. The results to date, indicate that cell bodies in the medulla project to all laminae in the lumbar dorsal horn and ventral horn; many fibers were found labeled with PHA-L in a golgi-like fashion. A small percentage (4.8±2%, n=5) of PHA-L-labeled terminals were labeled with serotonin immunoreactivity were found in each of the laminae 1-VII, VIII-X. Supported by NIMH grants NS13335, NS11255.


Previous studies have shown that the distribution of SST cells in the rat spinal cord overlaps with several areas receiving input from various putative neuropeptides including serotonin (5HT), substance P (SP) and leu-enkephalin (1-erik). In the present study the technique of retrograde transport was combined with immunohistochemistry to study the relationship between SST cells and chemically identified terminals in the rat spinal cord. Injection of fluorescent tracer (DAP or Fluoro-gold) was made into several different spinal cord areas. Following survival times of 4-11 days animals were perfused sequentially with saline and 4% paraformaldehyde, the spinal cords were processed for SST, SP and 1-erik immunofluorescence. Results of this study have shown SST, SP and 1-erik varicose fibers in the superficial laminae of the dorsal horn, nucleus proprius, the lateral neck of the dorsal horn, lamina X and the intermediolateral cell columns. Close apposition of immunofluorescent fibers were observed on large and small somata and on dendritic profiles. This work was supported by NIH grant NS19509 and by funds from the Miami Project Foundation.

SEROTONIN RELEASES ADRENOGENE FROM PRIMARY AFFERENT NERVE TERMINALS IN THE SPINAL CORD: POSSIBLE INVOLVEMENT IN SPINAL ANTIINOCICEPTION. M.S. Sweeney, T.D. White and J. Saboluk. Dept. of Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada, B3H 4HT.

Antinociception produced by intrathecal morphine and serotonin (5HT) is blocked by adrenergic receptor antagonists indicating that the antinociceptive effect produced by these compounds may be mediated by spinal cord serotoninergic systems. In some cases, rats were pretreated with capsicain either as neonates or adults and used in adrenocine release studies 17-20 weeks or 1 week later, respectively. 5-HT (50 µM) increased the release of endogenous adreno­ceptive of 5HT to spinal cord, but not to ventral, spinal cord synaptosomes. This release was reduced by the 5HT receptor antagonist methysergide, removal of Ca2+ from the medium, inhibition of scot-5' -nucleotidase, and both methods of capsicain pretreatment. These results suggest that activation of 5HT receptors on small diameter primary afferent terminals produces a Ca2+-dependent release of a nucleotide which is converted extracellularly to adeno­ne. This adreno­gene may contribute to the spinal antinociceptive effect of 5HT. (Supported by MRC Canada)


Intrathecal administration of morphine, and its antinociceptive effects were observed in a goldfish-like fashion. A small percentage (4.8±2%, n=5) of PHA-L-labeled terminals were labeled with serotonin immunoreactivity were found in each of the laminae 1-VII, VIII-X. Supported by NIMH grants NS13335, NS11255.


Serotonin (5HT) was administered intrathecally onto cat spinal cords while recording from WRH neurons in the spinal cord in vitro. The present study was to determine whether serotonin (5HT) also releases adrenaline from the spinal cord. Release of adrenaline evoked by 5HT from spinal cord synaptosomes was determined by HPLC with fluorescence detection of etheno-adrenaline. In some cases, rats were pretreated with capsicain either as neonates or adults and used in adrenocine release studies 17-20 weeks or 1 week later, respectively. 5-HT (50 µM) increased the release of endogenous adrenaline from dorsal, but not ventral, spinal cord synaptosomes. This release was reduced by the 5HT receptor antagonist methysergide, removal of Ca2+ from the medium, inhibition of scot-5' -nucleotidase, and both methods of capsicain pretreatment. These results suggest that serotonin stimulates the release of a nucleotide which is converted extracellularly to adeno­ne. This adreno­gene may contribute to the spinal antinociceptive effect of 5HT. (Supported by MRC Canada)
INTERACTION OF SELECTIVE OPIATE RECEPTOR AGONISTS AND AMITRIPTYLLINE ON NEURONAL RESPONSES TO PROLONGED SPINAL NOCICEPTIVE STIMULATION IN THE CAT.

This study examined the potential antinociceptive modulation of the combination of the mu-selective agonists DAGO or DPDPE and the noradrenergic reuptake inhibitor AMITRYLPATLINE (AMT) on spinal nociceptive neurons in cats. The effects of DAGO or DPDPE, AMT, or combinations of these agents were assessed on the responses of spinal nociceptive neurons to noxious stimuli. The results indicate that AMT and DAGO or DPDPE, either alone or in combination, significantly reduced the excitability of spinal nociceptive neurons, suggesting a synergistic antinociceptive effect. The mechanism of this effect involves the suppression of nociceptive input at the spinal level, with the combination being more effective than the individual agents.

Supported by the NIH Grant NR-09871

341.12 THE ROLE OF SEROTONIN (5-HT) AND NORPETHINEPHRINE (NE) IN THE ANALGESIC ACTION OF BETA-ENDORPHIN IN THE SPINAL CORD.

This study investigated the role of serotonin and noradrenaline in the analgesic action of beta-endorphin in the spinal cord. The effects of beta-endorphin on the release of serotonin and noradrenaline were examined in spinal cord slices isolated from rats. The results showed that beta-endorphin significantly increased the release of serotonin and noradrenaline in a dose-dependent manner. These findings suggest that the analgesic action of beta-endorphin in the spinal cord may involve both serotonin and noradrenaline pathways.

341.13 AMITRIPTYLLINE AND SPINAL ANTINOCICEPTIVE MECHANISMS.

This study examined the role of amitryptiline in spinal antinociception. The effects of amitryptiline on the release of serotonin and noradrenaline in the spinal cord were assessed in spinal cord slices isolated from rats. The results showed that amitryptiline significantly increased the release of serotonin and noradrenaline in a dose-dependent manner. These findings suggest that amitryptiline has a spinal antinociceptive effect that involves the release of serotonin and noradrenaline.

341.14 COCAINE SUPPRESSION OF MEDIAL THALAMIC NOCICEPTIVE RESPONSES IN THE RAT.

Recent behavioral experiments in our laboratory have demonstrated that cocaine (25 mg/Kg, i.p.) is a rapid-onset, non-opioid antagonist in the rat. The effect has been documented using the hot-plate and the formalin tests, and appears to be independent of cardiovascular or local anaesthetic actions of the compound. Antinociceptive doses of cocaine do not suppress spinal nociceptive reflexes although we have shown that at supraspinal levels, cocaine simultaneously enhances spontaneous activity but reduces noxious-evoked activity of caudally and rostrally projecting spinal modulation of neurons over a time course preceding the behavioral analysis. In the present study, we wished to determine whether cocaine would selectively alter nociceptively-evoked responses of neurons in the medial thalamus without affecting the spontaneous activity of nociceptive neurons in the lateral ventrolateral complex. Moreover, we investigated whether any observed effects were the result of thalamic or subthalamic mechanisms. Extracellular single cell recording of thalamic neurons in the anesthetized rat that was subsequently subjected to hyperalgesia showed that cocaine selectively decreased nociceptive responses of neurons in the lateral ventrolateral complex to noxious tactile stimuli without affecting the neuronal responses in the lateral ventromedial complex. The results suggest that cocaine's analgesic effect is mediated at least in part via a direct and selective suppression of medial thalamic nociceptive responses.
341.17

PAIN MODULATION: BIOCENIC AMINES

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Studies have demonstrated that serotonergic (5-HT) and catecholaminergic (NE) neurons in the spinal cord are involved in the modulation of pain. This suggests that drugs acting on these systems may be useful in the treatment of pain. In this study, we investigated the effects of chronic administration of 5-HT or NE on the expression of nociceptive withdrawal reflexes in the rat. Rats were trained to perform a conditioned avoidance response (CAR) in a habituation and test session paradigm. The CAR was measured as the latency to avoid a noxious stimulus. Rats were divided into four groups: control, 5-HT, NE, and combined 5-HT/NE. The results showed that chronic administration of 5-HT or NE decreased the latency to avoid the noxious stimulus, indicating a reduction in pain sensitivity. These findings support the hypothesis that serotonergic and catecholaminergic systems play a role in pain modulation.
HYPOTHALAMIC PVN STIMULATION PRODUCES ANALGESIA NOT MEDIATED BY VASOPRESSIN. O. R. L. Mathias, C. Johnston, and J. C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

The analgesic effect of hypothalamic paraventricular nucleus (PVN) stimulation and the involvement of vasopressin and opioid peptides in this process were studied in vasopressin-deficient (Brattleboro) and Long Evans rats. Rats were chronically implanted with a monopolar stimulating electrode in the PVN. Ten days after surgery, the animals were lightly anesthetized and PVN stimulation-produced analgesia (SPA) threshold was determined by the tail-flick test. Half the animals were then injected with naloxone (10 mg/kg) and half with saline. SPA threshold was determined again 20 min later. The same procedure was repeated on the following day, except that the drug assignment was reversed. PVN SPA threshold did not significantly differ between Brattleboro and Long Evans rats. Theimals were stable over the two sessions and was not significantly changed by naloxone. The result indicates that the analgesic effects of PVN stimulation are not mediated by either vasopressin or opioid peptides. Supported by NIH grant NS 07628.

EFFECTS OF DORSAL RAPHÉ, HABENULA AND EXTERNAL ELECTRICAL STIMULATION OF THE SEPTAL NUCLEI ON ANALGESIA IN THE RAT. W. D. Ong, M. Skolnick and N. Dafny. The Univ. of Texas Health Sc. Center at Houston, 77225.

It was recently proposed that most CNS structures which participate in a pain modulating mechanism will respond to noxious input, and when stimulated will induce analgesia (SPA). The mechanism underlying the SPA threshold has remained an open question. Several reports have suggested that electrical stimulation applied to the septal nucleus (SN) elicited analgesia. In order to test this hypothesis, we investigated the effects of focal stimulation of the dorsal raphe (DR) and habenula (Hab) nuclei, and external (ear) electrical stimulation (PES) on the septal nucleus of the rat. Twenty-five urethane-anesthetized Sprague-Dawley rats were used. Stainless steel bipolar stimulating electrodes were placed within the DR and Hab, and external electrical stimulating electrodes were inserted into the pinnas of each animal. Micropipettes (2M NaCl) were used as recording electrodes. It was observed that the septal nucleus cells exhibit 2 types of responses to noxious stimulation (tail pinch): "nociceptive-on" and "nociceptive-off". In addition, PES was more effective as anti-nociceptive than DR and Hab stimulation.

Supported by American Health Services Corporation, Inc.


External transcranial electrical stimulation (TCS) consisting of charge-balanced, rectangular, constant current (10-15 µA) low frequency pulses has been shown to produce endorphinergic analgesia in rats. In this report, we investigated the involvement of serotonergic involvement in TCS-induced analgesia. Subjects were 200 gm. male Sprague-Dawley rats naive to TCS but conditioned to handle and rest in the faraday cage. P-Chlorophenylalanine (pCPA) was injected to block the biosynthesis of serotonin and subjects were then tested for analgesia. The rats were conditioned and stimulated. Analgesia was assessed using a pressure tail flick test. Pressure (5 cm. from the tip) was exerted by a metal wedge mounted on a pneumatically driven syringe plunger. Maximum pressure tolerated by the rat was read as the rat made the first coordinated motor response to move its tail. This reading was averaged over four trials to produce the tolerated peak pressure (TPP). The difference in mean TPP before and after TCS was determined as a measure of analgesia. The experimenter was blind to the conditions of the experiment.

This 2x2 factorial design demonstrated a SHI dependent analgesic effect: pCPA blocked the TCS-induced analgesia. The TE treated, saline injected group showed a significant increase in mean TFP as all other groups—TE- MCPA, Sham-pCPA, sham-saline: N=62 p<.01 Tukey HSD.


Naloxone-reversible analgesia is induced by bilateral unilateral (ear) electrical stimulation of lesser amounts points on the ear (TCS). The present study tested the ability of thiorphan to potentiate analgesic effects of TCS by amplifying the effects of enkephalin release. Rats (n=20) were cannulated in the 3rd ventricle and implanted with electrodes through the apex of the annihil. Rats were pretreated for latency in the SPC wet tail flick test. Ten rats then received 30 min of TCS (10 Hz, 10 µA, charge-balanced rectangular pulses). Ten rats received 30 min. of "sham stimulation". Half of each group received 250 µg thiorphan iv in 100 µl 3A ethanol (5 /ul/min). The other half received injection vehicle alone. All rats were then restrained. Analgesia scores were increased in latency (sec.) from pretest, as shown below.

Effects of TCS and Thiorphan on Analgesia Scores for SPC TCS

THIORPHAN 3.4 ± 0.5* 1.9 ± 0.4
VEHICLE 1.0 ± 0.3 0.1 ± 0.3

N/ N/A reveals no drug and TCS effects, p<.01. The thiorphan - TCS group was different, p<.05, from all other groups. TCS analgesia may thus be mediated by endorphin release and intensified by enkephaline inhibition.

MODULATION OF CORTICAL SENSORY EVOKED POTENTIALS (SEP) BY STIMULATION IN NUCLEUS RAPHE MAGNUS (NRM) IN RATS. K.A. Poletz and G.P. Gebhart. Depts. of Neurosurgery and Pharmacology, Univ. of Iowa, Iowa City, Iowa 52242.

Midbrain electrical stimulation has been shown to reduce the amplitude of SEPs. Antinociceptive effects of midbrain stimulation are mediated via nucleus of the ventral medulla, including NRM. The purpose of this study was to determine whether SEPs could also be modulated by stimulation of NRM.

A conditioned test (CT) paradigm was used. Rats were anesthetized with Nembutal, N2O and O2, and paralyzed with pancuronium. The SEPs evoked by test stimuli (6 mA, 0.3 msec, bipolar needle electrodes delivered to the hindpaw) were recorded epidurally. Conditioning stimuli (CS) (10 pulses, 0.1 msec each) were delivered to the NRM through a monopolar electrode positioned stereotaxically. A adequate CS preceding the test stimulus consistently reduced the amplitude of the SEP. Threshold for the effect was as low as 25 µA with near complete abolition of the SEP by CS intensities of 50-100 µA. No latency changes were observed consistently. High CS pulse frequency (400 Hz) was more effective than lower frequencies. SEP reduction was most pronounced with CT intervals of 200-500 msec.

The attenuation of SEPs by NRM stimulation may occur through depression or modulation of the afferent, or occlusion at a cortical level, or by inhibition of other afferent pathways at subcortical levels.
342.9


Descending projections from several limbic regions terminate in the midbrain periaqueductal gray (PAG) and nucleus of the ventromedial medulla (VM). These pathways provide a substrate for the antinociceptive effects of limbic forebrain stimulation. The present study investigated the effects of limbic stimulation on intracellular responses of VM cells in the cat. Stimulation electrodes were placed in the central amygdaloid nucleus (ACE), the anterior hippocampus (AH), the bed nucleus of the stria terminalis (BST) and the septum, and the PAG. The final electrode placements were selected by minimizing the stimulation intensity required to suppress the tooth pulp-evoked jaw opening reflex. The majority of neurons in VM responded to PAG stimulation with a short latency, monosynaptic EPSP. Single shock or short train stimulation of ACE, the BNST, and AH also evoked mono- or oligosynaptic EPSPs in many of the same neurons. Cells excited by ACE or BNST were intracellularly stained with HRP. Labeled cells were large multipolar or medium fusiform neurons located in raphe magna or the adjacent magnocellular reticular formation. These results are evidence that analgesia associated with stimulation of limbic structures may be mediated by activation of antinociceptive pathways involving cells in the ventromedial medulla.

342.11

**ANTINOCESSION INDUCED BY MICROINJECTION OF CARBACHOL INTO THE VENTRAL PERIAQUEDUCTAL GRAY.** M. A. McCartney and N. K. Proudfoot. Dept. Pharmacology, Univ. Illinois at Chicago, Chicago, IL 60612.

The ventral rostral medulla (VRM) contains cholinergic terminals, muscarinic receptors, and microinjection of the cholinergic agonist, carbachol, directly into the n. raphe magnus (NRM) produces antinociception. However, the effects of carbachol microinjected into the n. reticularis gigantocellularis (NGC), n. gigantocellularis paragigantocellularis (NGP) and n. gigantocellularis (NGC) have not been evaluated. Therefore, studies were conducted to determine whether microinjection of carbachol into these sites produces antinociception. In addition, the role of endogenous enkephalins in mediating carbachol-induced antinociception was examined.

Microinjection of carbachol (2.5 ug) into the NRM, NGC, NGP, but not NRG, produced antinociception. However, this antinociception does not appear to be mediated by enkephalins since naloxone, administered either systemically or intrathecally, failed to reverse the carbachol-induced antinociception. This work was supported by USPHS Grant DA 03980.

342.13


GABA antagonists microinjected into the midbrain periaqueductal gray matter (PAG) or into the medullary nucleus raphe magnum (NRM) inhibits the nociceptive tail-flick reflex. Interestingly, this effect is due to the blockade of a tonic GABAergic inhibition of projection neurons from the PAG to the NRM, or from the NRM to the thalamus. The present study offers neural evidence for GABAergic modulation of midbrain/medullary antinociceptive pathways. For PAG studies, WGA-HRP conjugated to colloidal gold was microinjected into the NRM. In 55% of cases, enhanced, V-shaped sections of the PAG were embedded, thin-sectioned, and immunostained (on grids) with an anti-GABA-IR antibody. Approximately 40% of terminals in the PAG contained GABA-immunoreactive (IR) varicosities. These contain round vesicles, and form symmetrical synaptic contacts with dendrites and cell bodies, but not with axons. Approximately 50% of GABA-IR profiles are enkephalin containing. About half of synaptic contacts onto retrogradely labelled cell bodies are GABA-IR; none of these contacts were GABA-IR. For NRM studies, WGA-HRP was injected into the spinal cord, and an electrolytic lesion was made in the PAG. Approximately 48% of terminals in the NRM are GABA-IR and contain both GABAergic and enkephalinergic synaptic inputs to dendrites and cell bodies. About 3% of GABA-IR terminals are asynaptically contacts and contained flat varicosities. Degenerating PAG terminals and GABA-IR terminals converge onto raphe-projecting neurons. These data indicate there are multiple sites of GABAergic modulation of descending antinociceptive control. Supported by NS16267 and NS16455.

342.10

**EFFECTS OF SINGLE AND CONJOINT MET-ENKEPHALIN MICRO-INJECTIONS IN RAT CENTRAL GRAY (PAG) AND NUCLEUS RETICULATUS PAGINALIS (PAG).** A. S. I. Sil, and R. S. Bickel. Pain Physiology Laboratory, Mass General Hospital, Boston, MA 02114.

As stated in data, 15 neurons histologically identified as being within the VM nucleus of 38 rats were studied. The rats were anesthetized under 55 mg/kg Pentobarbital for surgical preparation, and then maintained in light anesthesia (with 20% initial dose per hour, IM) adequate to allow tail flick reflex > 70% of the cases. Wound margins were locally anesthetized with lidocaine. Rats were maintained at 36-38°C and normal temperatures. Neurons showing spontaneous activity were stimulated intra-axially with brief (2-4 sec, 0.1-0.5 mA) 100 Hz anterior spinal antinociceptive stimulation between left T9 and right T10 dorsal surface of spinal cord, followed with collision testing. Only 8 rats satisfied the RMS criteria. Spontaneous activity of 7% of the RM cells was augmented by light anesthesia (20%). This was followed by no response. Noxious heat enhanced spontaneous firing in 56% of the neurons, inhibited in 37%, and had no effect in 6%. Noxious pinch had similar effects. (Thus 56% ± 37% ± 3% + 4% = 100%). Considering all 57 RM cells, single and conjoint Met-Enkephalin injections of PAG (2 min, 200 nl, 10 ug) and POC (2 min, 100, 5 ug) produced enhancement of spontaneous activity (>50%) in about 3/5 of the tests and depression in 2/5 of the rats. The most significant finding was that the effect of enkephalin iontophoresis could be predicted largely by the effect of noxious stimulation. In those 32 PAG RM cells whose firing levels were enhanced by noxious stimulation, 100% (18 of 18) were enhanced by PAG injection, and 97% (24 of 25) were depressed by conjoint injection. In those 35 RM neurons whose firing rates were depressed by noxious stimulation, 100% (8 of 8) of the rates were enhanced by PAG injection, 90% (9 of 10) were enhanced by POC injection and 100% (16 of 16) were enhanced by conjoint injection. These patterns obtained for RM as well as RM neurons. (Supported by NSF grant DE 07905.)

342.12

**BEHAVIORALLY DERIVED REFRACTORY PERIOD ESTIMATES OF THE SUBSTRATES FOR ANALGESIA DERIVED FROM STIMULATION OF THE DORSAL AND VENTRAL PAG.** Susan Schenk and Kim Polland-Smith*. Texas A&M University, Dept. Psychology, College Station, TX 77843.

Physophysical methods were used to obtain refractory period estimates of the directly stimulated substrate for the analgesic effects of periaqueductal gray (PAG) stimulation. Trains of stimulation pulses (10 sec, train length, 0.1 msec monophasic constant current cathodal pulses) were delivered to the dorsal or ventral PAG of restrained rats. Immediately following the stimulation, the caudal 2.5 - 3.0 mm of the rat's tail was immersed in heated water (52 - 54°C) and latency to tail flick was measured. The refractory interval was determined as the frequency of stimulation that resulted in a tail flick latency longer than 6 sec. Pairs of stimulation pulses were also delivered at interstimulus intervals of 1.0 - 10.0 msec. Frequency thresholds for analgesia under this stimulation condition were compared to the threshold when only single pulses were delivered. Results indicated that the effectiveness of the paired pulse stimulation increased gradually as pulse-pair interval was increased from 1.5-7.5 msec for both the dorsal and ventral sites. These data suggest that the analogic properties of stimulation derived from dorsal or ventral PAG sites rely on the direct activation of similar caliber neurons.

342.14


The present study was designed to determine the location of brain stem neurons that receive direct input from the periaqueductal gray (PAG) and project to the spinal cord. Ten adult Sprague-Dawley rats received multiple injections of 4% Fluoro-gold into the lower 3.0 mm of the spinal cord and a single iontophoretic injection of phoxolus vulgaris leucoagglutinin (PHA-L) into the midbrain PAG. Following fixation, the brains were sectioned and PHA-L labeled fibers were visualized using immunofluorescence. The mean number of retrogradely labeled neurons (per 0.04 mm² area per side) that were contacted by PHA-L labeled fibers were quantitated. The nuclei containing the greatest number of spinobulbar neurons, which appears selectively innervated by PHA-L labeled PAG projection neurons, were the gigantocellular reticular nucleus per alpha, nucleus subcoeruleus, lateral paragigantocellularis nucleus, pedunculopontine tegmental nucleus, oral pontine reticular nucleus, ventral gigantocellular reticular nucleus, ventrolateral tegmental nucleus, raphe magnus and rostroventromedial reticular nucleus. These results suggest that several brainstem nuclei may relay PAG input to the spinal cord and quantitatively, the raphe raphe magnus is the most significant. Supported by NSF grant BNS-8607520 and NIH grants NS 19208, DE 06682 and DA 04090.

We have used in situ hybridization to define the expression of preproenkephalin and preprodynorphin genes in neurons of lamina I and II of the trigeminal nucleus caudalis.

Neurons expressing preproenkephalin were more likely to be found in lamina I and the outer layer of lamina II; neurons expressing preproenkephalin were more uniformly distributed within laminae I and II.

After lesions of the trigeminal ganglion, neuronal expression of preproenkephalin decreases, due to the decline in the number of neurons expressing this gene, while the number of neurons expressing preprodynorphin increases.

0.1mA stimulation of the trigeminal ganglion increases the number of neurons expressing preproenkephalin, while preprodynorphin mRNA expression in the same animals and with electrostimulation of the PAG shows a more complex pattern of changes.

These results support specific patterns of opioid peptide gene regulation by both primary afferents and descending inputs from the PAG, and provide examples of opposite function-related changes in expression of the two principal brain opioid peptide genes.

HIPPOCAMPUS AND AMYGDALA III

AMygDAla DIRECTLY INNERVATES BRAINSTEM CATECHOLAMINERGIC CELLS IN THE RAT. T.S. Gray and D.J. Magoun*. Dept. Anatomy, Stritch School of Medicine, Haywood, IL 60153

The present study used a combined phasolus vulgaris leucoagglutinin lectin anterograde tracer (PHA-L) and immunocytochemistry to determine whether amygdala cells directly innervated tyrosine hydroxylase, PNTM, and/or dopamine beta hydroxylase immunoreactive cells within the brainstem. Isotophoretic injections of PHA-L were placed within the central nucleus of the amygdala of anesthetized 150-250g Long-Evan rats. Two weeks later animals were overdosed with sodium pentobarbital and their brains were fixed through vascular perfusion. Amygdaloid terminals were demonstrated by antibodies to PHA-L with avidin-biotin immunocytochemistry and using a brown detection system. Catecholaminergic cells were visualized via antibodies to TH, PNTM or DBH using a glucose oxidase-nitro blue tetrazolium reaction. Amygdaloid terminal were distributed in a variety of areas that were immunoreactive to catecholamine markers. For example, amygdaloid terminals appeared to contact tyrosine hydroxylase immunoreactive cells within the substantia nigra, locus coeruleus and the A8 cell group. Amygdaloid terminals also appeared to contact TH and PNTM immunoreactive cell bodies within the nucleus of the solitary tract and the ventrolateral medulla. The results demonstrate that the amygdala can directly influence the number of subpopulations of catecholaminergic neurons within the brainstem. (Supported by NIH NS 20041)

VIDEO MICROSCOPY OF CULTURED AMYGDALA BRAIN SLICES. D.R. Stevens*, E.W. Knecht, P.F. Chapman*, and T.H. Brown, SPON: C.L. Kornan) Division of Neuroscience, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Long-term synaptic potentiation (LTP) was recently discovered to occur in synapses of the acute amygdala brain slice (P. Chapman and T.H. Brown, this meeting). To increase the accessibility of this complex structure to high-resolution analysis of such synaptic phenomena we have applied a simple microphysiological procedure, which was previously developed (Gahwiller, B.H. J. Neurosci. Meth. 4:329, 1981) for use on hippocampal tissue.

Horizontal slices of amygdala, including (several regions) cortex, fundus striati, dorsal dentiform nucleus, lateral hypothalamus and subiculum) were prepared from the brains of 6-8 day old rats previously perfusion-fixed with formalin. The slices were cultured on collagen-coated coverslips and maintained for periods of up to several weeks. Our preliminary studies have focused on the development of the cultures and the morphology of synaptic transmission. The synaptic cytoarchitecture and cellular morphology were examined using video-enhanced differential-interference contrast (VEC-DIC) microscopy. By 2 weeks in vitro the cultures had flattened into a 1-4 cell layer structure. Low-power (4X10X)VEC-DIC microscopic observation of the living slices and of cresyl violet stained slices revealed the presence of recognizable amygdaloid nuclear regions as well as cortical cell layers. When viewed at higher power (40X) magnification, individual neuronal somata were readily visualized in the living tissues. Intracellular injection of yellow fluorescent dye in some neurons revealed unusual neuronal morphologies that were similar to those seen in published cortical materials.

We conclude that the cultured amygdala slice preparation holds great potential for combining biophysical and neuropharmacological analysis with visualization techniques such as VEC-DIC microscopy and confocal scanning laser microscopy. These combined approaches should allow the study of the development and microphysiology of synaptic transmission in this important but complex temporal lobe structure. (Supported by AFOSR and Beckman Research Institute)
343.3
STIMULATION OF THE AMYGDALOID CENTRAL NUCLEUS (ACe) FACILITATES THE RETROGRADE MEMBRANE RECONSTITUTED MEMBRANE CONDITIONED REFLEX (MNR) IN THE RABBIT. P. J. Whalen and B. S. Kapp. Dept. of Physiology, Univ. of Vermont, Burlington, VT 05405.

The median of the MNR during Pavlovian conditioning appears to involve an associative learning process (Weisz and McInerney, 1987). Since the ACe (a) contains various reflexes (Gary Bobo and Bonvallet, 1975; Schlor et al., 1984; Pascoe et al., 1987), (b) projects to the brainstem (Kapp et al., 1986), the present study was conducted to evaluate the contribution of the ACe to the facilitation of the MNR.

New Zealand rabbits were prepared with stimulation electrodes (100um tip) in the ACe. Following recovery, the MNR was elicited over 16 trials. Electrical stimulation of the ACe, at an intensity which influenced the brainstem as indexed by vagal bradycardia, was presented for either 200 or 100msec prior to reflex elicitation for eight of these trials (1000uamps, 100Hz). A significant increase in reflex amplitude (10-24%, p<.05) was observed on ACe stimulation trials when compared to trials not preceded by stimulation. Stimulation of the ACe did not induce membrane movement. These results are consistent with the notion that the ACe may contribute to associative facilitation of the MNR.

343.5

We have compared the distribution of basal forebrain (BF) afferents with muscarinic receptors and putative presynaptic cholinergic markers in the hippocampal formation (HF) and entorhinal cortex (EC). BF afferents to the HF and EC were labeled using anterograde transport of [3H]-labeled amino acids. Muscarinic receptors were labeled with [3H]-pirenzepine (M1) and [3H]-quinuclidinyl benzilate (M2). AChE, hemicholinium-3, which binds to high affinity choline uptake sites (HACU), were used as putative presynaptic markers. In the HF the distribution of BF afferents, AChE and HACU showed close correspondence except in stratum molecularis (SM) of the dentate gyrus (DG) where BF afferents and HACU were uniformly distributed but AChE reaction product was most dense in the inner 1/3. Like BF afferents, AChE and HACU were distributed densely in area orbi and pyramidale of CA1-4 and in the prosobulbar. However, M1 and M2 sites did not follow the distribution of BF afferents, AChE, or HACU sites in several areas. For example, in SM of the DG M1 sites were very dense compared with BF afferents, M2 and HACU. In the subiculum, M1 sites were very dense compared with BF afferents. AChE and HACU in EC, BF afferents, AChE, HACU and M1 sites showed small laminar variations in distribution, but M2 sites were dense in layer III, especially in area 28S. In the HF, M1 and M2 sites were the good markers for BF cholinergic afferents except in the inner 1/3 of SM of the DG. In the subiculum and layer III of EC, M2 sites are very dense while other cholinergic markers are not. (Supported by NS19416, A000061, AO43621 and T22-N07152).


Hippocampal (HIPP) stimulation significantly decreases heart rate and blood pressure, and we hypothesize it does so via its connection with the medial frontal cortex (MFC), a region which projects directly to the solitary nucleus (NTS). The present electrophysiological and anatomical study was undertaken to determine the degree to which the HIPP projection to MFC overlaps the origin of the descending projection to NTS.

72 MFC neurons were antidromically activated by HIPP stimulation with an average latency of 31 msec (SD = 10). HIPP neurons responded to HIPP stimulation in the following order: in a few cases, the orthodromic spike from HIPP collided with and eliminated the antidromic spike from the NTS. Following injections of anterograde and retrograde tracers, serial sections adjacent to those processed to reveal the HIPP projection to MFC were processed to reveal the terminal distribution of the retrogradely labeled cells from NTS in the MFC. Electron microscopy confirmed that HIPP-labeled terminals do, in some cases, synapse on HIPP-labeled cells. These results indicate that the pathway from HIPP to the MFC cells which project to NTS may be monosynaptic and, therefore, directly influence a central vegetative control system. (Supported by Loyola University Potts Estate Fund Grant 84-249).

343.4

Injections of anterograde and retrograde tracers were made in different sectors of the prefrontal cortex (PFC) in cats and macaques, and the resulting labeling in the hippocampal formation was analyzed. Serial sections adjacent to those processed to reveal the transported tracers were stained for Acetyl Cholinesterase (AChE) histochemistry. Our results indicate that only the dorsolateral sector of macaque PFC projects intensely to the presubiculum. In cat, the ventral PFC projects to the parasubiculum. The terminal labeling in this region is densest in the deep part of layer I, and overlaps with an intensely stained AChE band. In both cat and monkey, the subiculum projects heavily to the ventral PFC. All prefrontal sectors studied are connected with the so called caudomedial lobule in macaque and caudomedial band in cat.

These findings suggest that the connections of the PFC with the hippocampal formation exhibit notable similarities in the two species studied. This argues in favor of the existence of comparable functions of the prefronto-hippocampal loops. However, connectional differences are also present, which may entail functional implications. (Supported by Grants 0512/84 from CAICYT and PB86-0110 from CICYT).

DISTRIBUTION OF CORTICAL PROJECTIONS TO THE MONKEY ENTORHINAL CORTEX: AN AUTORADIOGRAPHIC STUDY. FOR. INSUASTI, AND. D. G. AMARAL. The Salk Institute, P.O. Box 8580, San Diego, CA, and Dept. Anatomy, Univ. of Navarra, Pamplona, Spain.

Previous retrograde tracing experiments demonstrated that the macaque monkey entorhinal cortex receives several direct neocortical inputs (Insuasti et al., 1987). In order to analyze the topographical distribution of these projections, discrete injections of [3H] amino acids were made into several of the anterior cortical areas. Orbifrontal and temporal polar cortical regions project relatively widely within several subdivisions of the entorhinal cortex. More restricted projections were observed from the perirhinal cortex (which terminally innervates the rostral half of the entorhinal cortex), and the superior temporal gyrus, parahippocampal gyrus, and the retrosplenial cortex (all of which preferentially innervate the caudal half of the entorhinal cortex). The lateral field of the entorhinal cortex generally receives a heavier cortical input than other entorhinal fields except when the injection involved the retrosplenial cortex. In this case, heavier labeling was located medial and caudal to the lateral subdivision. In all cases, layer I received the densest terminal innervation, followed by layers III, V and VI. These results indicate that the cortical projections to the entorhinal cortex are diffusely distributed but have a rough topographic organization.

343.8

Hippocampal stimulation inhibited subical (SP) output neurons to the PPN. While microinjection of MMS into the hippocampus increased locomotor activity, suggesting that signals from the hippocampus disinhibit PPN neurons to produce hypermotility (Yang & Mogenson, Neurosci., 23:1041-1055, 1987). In contrast, microinjection of MMS into the amygdala suppressed locomotor activity which was also mediated via the SP (Yin & Mogenson, Proc. Can. Fed. Biol. Sci., 30:120, 1967). It was hypothesized that these effects were due to differences in connections between the amygdala and hippocampus and the PPN. In a total of 50 SP-PPN neurons, only 20 were able to be tested with single pulse stimulation of the amygdala and hippocampus. Fourteen were activated by amygdala stimulation and 6 were inhibited whereas 5 were activated by hippocampal stimulation and 13 were inhibited (X2=5.17, p<.05). These observations provide electrophysiological evidence that the amygdala and hippocampus have opposite effects on SP neurons projecting to the PPN. (Supported by NSERC of Canada).
343.9

In cat, the behavior of attentionless focused attention upon a target is accompanied by the development of rhythmic cortical activities at 36 Hz ("beta" rhythms) in the fronto-parietal cortex. Both behavior and accompanying rhythms are controlled by the ventral tegmental area (VTA): bilaterally VTA lesioned cats display hyperexcitability in conditions in which normal animals would develop immobility and watching (Moneta et al., Behav Brain Res. 6:129, 1982). On the other hand, bilateral lesion of nucleus accumbens (Nac), a relay on the ventral striatal pathway from VTA which projects to the striatum has the opposite effect, eliciting perseveration of movementless attention-fixated with a high rate of cortical beta rhythms (Bouyer et al., Exp Neurol. 92:689, 1986). The VTA is also a source for connections to the amygdala which in turn projects to Acc. With these data in mind, we have now performed bilateral kainic lesions restricted to the basolateral nucleus of the amygdala and controlled the postlesional behavior and beta activity. Both manifestations were markedly decreased after such lesions, but interestingly, no spontaneous motor hyperactivity could be noticed in this condition.

From this we tend to conclude that in our experimental conditions the amygdala controls the motivational component of the attentive state with no major effect upon its motor behavior component, at variance with the Acc, which being part of the neural complex and also receiving amygdala influences, may exert a higher level control on attention. Supported by DRET (N°86-101) and Fondation pour la Recherche Médicale.

343.11
BASAL FOREBRAIN AFFERENTS TO THE HIPPOCAMPAL FORMATION IN THE RHESUS MONKEY. D.L. Rosene, P.L. Heilbroner and M.B. Moss, Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Projections from the magnocellular basal forebrain nuclei to the hippocampal formation (HF) were examined using acetylcholinesterase (AChE) histochemistry and anterograde and retrograde tracers. The medial septal nucleus (MS) and vertical limb (VSD) of the diagonal band (VDB) projects to uncinate levels of the HF, especially to laminae of CA4, CA3, CA2, and the prosubiculum (ProS) as well as the molecular layer of the dentate gyrus (DG). This projection is distributed throughout the entire length of the HF but was progressively diminished caudally. Like the MS-VDB, the nucleus basalis (NB) projects most heavily to the uncal HF, although less densely. Only dense NB projection was to the border of the stratum molecular and stratum radiatum at the junction of CA1 and ProS. These projection patterns correspond closely to the laminar pattern of AChE except in the molecular layer of the DG where the basal forebrain afferents were uniform.

Injections of retrograde tracers into the HF confirmed that these projections originate from MS-VDB and NB. AChE double-labeling demonstrated that from 30 to 50% of the retrogradely labeled neurons were likely to be cholinergic.

(Supported by NS19416, AG04321 and NS07152).

343.13
IDENTIFICATION OF ZINC-CONTAINING NEURONS BY RETROGRADE TRANSPORT OF ZINC-SELENIDE. C. A. Howell and C. J. Frederickson, Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, Texas 75083.

We previously have shown with lesion techniques that the zinc-containing terminals in the BST, N. terete, and VM arise from axons of the stria terminalis and fornix/fimbria (Frederickson et al., Soc. Neurosci. Abst., 1986, 12:1532). In the present work, we extended the ZnSe technique as a transport marker for zinc-containing neurons (Danscher, In: Frederickson et al., The Neurobiology of Zinc, Liss, N.Y, p. 180). Here we sought to identify ZnSe positive cells of origin of these zinc-containing pathways.

Selenite ions (Na2SeO3) were infused into BST/VMH regions in anesthetized rats, cauterized to precipitate ZnSe in situ in zinc-containing terminals. Twenty-hour later, rats were sacrificed, and cryostat sections were developed in the dark, rendering the ZnSe visible by silver encapsulation.

Staining at the injection site showed characteristic labeling of the neuropil (presumably the zinc-containing boutons) with no labeling of perikarya. However, dense and selective labeling of individual perikarya was found in the lateral amygdala, the amygdaloid nuclei, and in the ventral subiculum. These findings suggest that retrograde transport of precipitated zinc (ZnSe) is a chemospecific marker for identifying and mapping zinc-containing neuronal systems. Supported by NIH 42796.

343.10
ORGANIZATION OF HIPPOCAMPAL EFFEENT PROJECTIONS TO THE CEREBRAL CORTEX IN THE RHEAS MONKEY. G.J. Blat and D.L. Rosene, Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

To identify the cells of origin of the direct hippocampal formation (HF) projection to the cerebral cortex, injections of retrograde fluorescent tracers (Phosphine gold (PHG) and anterior (APHG) para phosphine gold) and the medial (MFC) and orbital frontal cortex (OFC). Injections in the medial PHG labeled cells in the subiculum and through a central strip of CA1 stratum pyramidale. This strip extended longitudinally the entire anteroposterior length of the HF. Similarly an injection placed laterally in the PHG labeled a longitudinal strip in the most lateral part of CA1 while injections in the APHG lateral to the rhinal sulcus labeled cells in a more medial strip of CA1. In contrast, MFC and OFC injections labeled cells mainly in the subiculum while in CA1 labeled cells were widely distributed but limited to the deepest part of stratum pyramidale. These results demonstrate that projections from CA1 to the PHG and APHG originate topographically from longitudinally oriented strips of cells in contrast to projections to the MFC and OFC that originate from the deepest part of stratum pyramidale. These observations suggest a unique functional differentiation in CA1 of the monkey HF.

(Supported by NS19416, NS16841 and AG04321)

343.12

Electrical stimulation of the dorsomedial-posterior hypothalamic nuclei in the unanesthetized rat produces theta (θ) in the hippocampal formation. Furthermore, increases in stimulus intensity are linearly related to increases in θ frequency. This effect is mediated by the medial septum/diagonal band nuclei since after lesions here θ can no longer be produced in the hippocampal formation by such stimulation. The purpose of this study was to investigate the effects of dorsomedial-posterior hypothalamic stimulation on the discharge properties of medial septal/diagonal band cells. Cells were classified along three dimensions, according to previously used criteria (Colom and Blang, Brain Research, 1987), as theta on or off, phasic or tonic, linear or non-linear, during the spontaneous occurrence of θ and large amplitude irregular activity (LIA). Stimulation did not affect a cell's classification as on or off or phasic or tonic. The major effect was to change phasic non-linear theta-on cells to linear, suggesting that this ascending hypothalamic system plays an important role in increasing the number of septal cells that code the frequency shifts of hippocampal formation theta.
COLOCALIZATION OF GABA AND PEPTIDES IN THE RAT BASOLATERAL AMYGDALA. A. J. McDonald and J. C. Pratesi
Departments of Anatomy, Univ. of South Carolina Sch. of Med., Columbia, SC 29080, and Wright State University Sch. of Med., Dayton, OH 45565.

Although previous studies have shown that GABA and peptides are found in nonpyramidal neurons of the basolateral amygdala, it is unknown whether these substances co-exist in the same neurons. The present study utilized a two color ABC immunoperoxidase procedure (with DAB and BODIPY as chromogens) to investigate this question. The anti-GABA antiserum was raised in a guinea pig, while the antibodies to peptides were raised in rabbit. Most somata of GABA-positive and neuropeptide Y-positive neurons were GABA-positive. All large CCK-positive neurons appeared to contain GABA while a subset of small CCK cells, found mostly in the lateral nucleus, were mostly GABA-negative. Some VIP containing neurons also contained GABA. Thus, it appears that many neurons in the basolateral amygdala contain the inhibitory transmitter GABA also contain putative peptide neuropeulators. The data suggest, as in other regions, that GABA and neuropeptides are co-localized in the same neurons and act in concert.

Supported by NIH Grant NS 19733.


Very little information is yet available on the localization of neurotransmitters in the amygdala of primates. This study will present results on the peroxidase-antiperoxidase immunohistochemical method used to stain serial coronal sections of the amygdala (Saimiri sciureus) with polyclonal antibodies to tyrosine hydroxylase (TH), serotonin (5-HT) and met-enkephalin (ENK), and with a monoclonal antibody to substance P (SP) bodies were noted in the basolateral nucleus and, more rarely, in the central nucleus of the amygdala, while terminal labeling or a minor small caliber occurred in the region of the central nucleus as well as in the central and basolateral nucleus. Numerous ENK-immunoreactive fibers of the woolly type were seen in the lateral central and basolateral nucleus and, to a lesser extent, in its medial portion which also contained many thinner axons and scattered terminals. 5-HT immunostaining in the amygdala was widespread with varicose fibers being particularly prominent in the basal, lateral and central nucleus. 5-HT terminals were visualized in the central nucleus of the amygdala and the periamygdaloid cortex. TH immunoreactivity was seen mainly as scattered terminals in the central nucleus and fine varicose fibers in the lateral and basal nucleus. The present study provides the first description of the distribution of SP, ENK, 5-HT, and TH in the amygdala of primates. These results should serve as a more rational basis for defining amygdaloid nuclei in mammals and as a framework for future studies of the chemospecific connections of the amygdala in primates. (Supported by grants of the MRC, FRQ and FCAR).

MEMORY FIELDS: DIRECTIONAL TUNING OF DELAY ACTIVITY IN THE DORSOLATERAL PREROFONTAL CORTEX OF Rhesus Monkeys. S. Furnah, C. J. Bruce, and P. G. Goldman-Rakic

The prefrontal cortex (PFC) in nonhuman primates is essential for the spatial working memory processes tapped by delayed response tasks. Our previous study using an occluding operant paradigm provided evidence that PFC neurons are active (excitation or inhibition) in the delay period only when visual cues were presented at specific locations in the visual field (Furnah et al. Soc. Neurosci. Abstr. 12:554, 1986). We report the area of the visual field from which single unit delays activity as that neuron's memory field. In the present study, we quantitatively analyzed the spatial tuning of these memory fields. Eight visual cues separated by 45 deg in polar orientation at an eccentricity of 12 deg were presented randomly in the cue period of the occluded delayed response task. Tuning curves were based on the average discharge rate for 300 ms during the delay period for each cue. The best directions were estimated from the parameters used for the fitting experimental data to a Gaussian function. Tuning indices were defined as the departure from the best direction which reduced the response by 50%. Tuning indices were made based on the average discharge rate between 20 and 40 deg and 40 deg and 60 deg. Our results indicate that PFC neurons have a range of memory fields, with each hemisphere primarily mapping the contralateral hemisphere. The findings that most memory fields are comparable to visual receptive fields and movement fields in the posterior parietal cortex and the superior colliculus suggest that the PFC is closely linked to the brain's centers for spatial vision.


Lesion of the monkey hippocampus produces spatial memory deficit. Neuronal activity in the monkey hippocampus and entorhinal cortex was recorded during operant feeding, drinking and shock avoidance, delayed matching the visual or the auditory, and a clinical test with visual, auditory presented from various directions. Focus in the clinical test was on neuronal response selectivity to direction. Of 1075 neurons tested, 581 (54.0%) responded to some task or the clinical test and 399 (37.1%) were identified as hippocampal type, 40 (3.7%) were direction specific and many of these were in the caudal hippocampal formation. Of these 40 neurons, 27 responded only to visual stimuli (left anterior, 4; right anterior, 22; multidirectional, 1), 8 responded only to auditory stimuli (anterior, 1; right posterior, 2; posterior, 3) and 5 responded to both visual and auditory stimuli (right anterior, 1; multidirectional, 4). The direction-selective neurons tended to respond to visual stimuli presented from the right anterior, and to auditory stimuli presented from the posterior direction. The results suggest hippocampus and entorhinal cortex involvement in the central processing of directional or spatial information.

CHANGES IN HAND KINEMATICS DURING A QUANTIFIABLE LEARNING PARADIGM IN PRIMATES. C. L. Ojakangas, D. K. O'nostro, D. C. Tam and T. J. Done.

A paradigm involving a 2-dimensional, visually guided arm movement task was used to provide a criterion for hand movement control. A manipulandum on a video screen from a start box to one of 4 randomly presented target boxes. The relationship between hand and cursor was the same for the control phase (1.0) and was varied during learning. Movement strategies during adaptation to 4 gains (.6 - 2.0) were studied via control, learning and testing phases. Results indicated that learning a new gain monkeys scaled the velocity of their movement to match the distance traveled (i.e., further distances required higher velocity) (p < .01, 2-tailed t test). Time to maximum velocity remained relatively constant over phases and across gains (p = .01), as did reaction time. With time constant, distance until first (maximum) velocity peak varied directly with gain change. During learning, the 2nd velocity peak seen initially was eliminated and hand trajectory smoothed. Total movement duration decreased (p < .01) although remained greater than for smaller gains once learned (p < .01). Results support the hypothesis that movement duration is a free variable and invariant during the first phase of movement. The learning strategy consists of scaling hand velocity to match gain. Supported by NSF/BNS 8707572 and NIH/NS 18338.

CATHECOLAMINE SENSITIVITIES OF PREFRONTAL NEURONS RELATED TO A DELAYED RESPONSE TASK OF BERRIES. S. Sawasuchi, M. Matsumura and K. Kubota.
Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Influences of iontophoretically applied dopamine (DA) and noradrenaline (NA) on neuronal activities related to a delayed response task were examined in the prefrontal cortex. The task was started by monkeys rotating a handle to a central zone, and consisted of pre-cue (central lamp, 1 s), cue (green lamp, 1 s), delay (4 s), go (red lamp, rotating the handle to left or right zone within 1 s), and reward periods. Prefrontal neurons showed changes in activity during the pre-cue period (P1-types, n=16), both the cue and go periods (Cue/Go-types, n=16), the go period (Go-types, n=16), and the delay period (Delay-types, n=16). Delay-types consisted of Differential neurons (n=33) whose activity differed between left- and right-cue trials, and Non-Differential neurons (n=31). DA increased the activity of most of Cue/Go- (16/16), Go- (13/16) and Delay-types (49/64), and NA inhibited the activity of most of PC-types (13/18) and Non-Differential Delay-types (25/31). Dopamine and haloperidol attenuated changes in activity during cue, delay and go periods, while sulpiride had no clear effects. Results suggest that DA prefrontal neuronal activity involved in temporal integration of visual cue and motor performance, and NA inhibits prefrontal neuronal activity related to visual reception.

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HIPPOCAMPUS AND AMYGDALA IΠ
LEARNING AND MEMORY: PHYSIOLOGY IV
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Animal studies have shown memory enhancement following post-trial administration of intermediate doses of glucose as well as an increase in the amplitude of the ERP word repetition effect, when the animals were administered 30% or 100 g glucose showed negative correlations between BCG and recognition scores (p's < 0.05). These results confirm the importance of glucose in memory modulation.

SHORT AND LONG LATENCY BLINK CRs ARE SUPPORTED BY ACTIVITY OF UNITS IN THE MOTOR CORTEX AND THALAMUS OF CATS. C.D. Woody, S. Aou, E. Grum, S. Birt, O. Melamed, and J. Wangwongvivat, UCLA Med. Ctr., Los Angeles, CA 90024. Recent ablation studies suggest that long latency eye-blink conditioning depends on cerebellum and subcerebellar nuclei for its development. We examined the hypothesis that higher brain structures might support such conditioning in normal, intact animals. Recordings from the motor cortex of awake cats disclosed patterns of unit activity that increased to support performance of Pavlovian blink conditioning. The activity in the cerebellar nuclei also found increases in activity of short and long latency correlated with development of conditioning. Further studies demonstrated the role of short latency activation of single units of the motor cortex after pairing click CS with tap US and local inophoretic application of glutamate. Activity in the cerebellar nuclei was increased in neuronal excitability and input resistance and a decrease in a current resembling K+ measured by single electrode voltage clamp. Ablation of the motor cortex of cats prevents short but not long latency blink conditioning. (Supported by NS25510/HD05958.)
LEARNING-RELATED CHANGES IN SINGLE-UNIT ACTIVITY IN THE MEDIAL TEMPORAL LOBE (MTR) OF RABBITS. L. M. Gibb, J. J. Karim, and R. M. Weinberger. (Supported by VA Institutional Research funds)

The present study was concerned with thalamocortical, hippocampal and auditory neuronal systems underlying auditory working memory in the rat. The task was an auditory continuous nonmatching-to-sample (Sakurai, Psychobiol. 15:577, 1987). Preserving a panel (G) was rewarded if the tone for the current trial was different from the tone for the preceding trial. A 3 s of delay period was imposed between the tone presentation period on preceding trials. The entorhinal cortical (EC) and the dorso medial thalamic (DMT) units showed differential response according to the one of the tone (high or low) and outcome of the next response (correct or error) respectively. In the delay period, the DMT units showed differential activity related to the outcome of the next response. In the tone-presentation periods immediately prior to response, the medial genulate body (MGB) units showed tone-differentiated activity. The MGB, CA1, CA3, EC, dentate gyrus and motor cortex had more units showing differential activity related to type of the next response (Go or No-Go). A macro neuronal model underlying the auditory working memory will be discussed. (Supported by Grant-in-Aid for Scientific Research 62170339 from the Japanese Ministry.)

SHORT TERM MEMORY PROCESS IN THE PARIETAL EYE. D. J. Eder and R. K. Radar. (Supported by ONR N00014-87-K-0433 and Monsanto.)

The present study was concerned with the development of discriminative bradycardiac CRs in rabbits (Buchanan SL and DA Powell: JCP Ph 78:567, 1976), and the CS-evoked multiple activity in the PFCm under conditioning-trained-induced changes that are reliably correlated with averingly sophisticated changes in heart rate (Gibbs CM and DA Powell: Brain Res 442:66). Accordingly, we have begun evaluating single-unit activity in the PFCm during or following either simple Pavlovian conditioning (tone CS paired with eyeshock) or differential conditioning (one of two tones paired with eyeshock) or nonassociative training (e.g. unpaired stimuli). Our results to date have indicated the PFCm consists of numerous, widely interconnected cell populations differing from one another with respect to the patterns of their interaction. CS and US-evoked activity. Our data have also suggested that at least three of these functional populations show associative training-induced changes in their CS-evoked discharge that are consistent with the concomitant development/expression of bradycardiac CRs. These data thus provide further support for the suggestion that the PFCm participates in learning and cardiovascular adjustments in rabbits. (Supported by VA Institutional Research funds)

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A reliable source of uniform random numbers is an essential building block for any computer simulation program involving a stochastic process. Many system supplied pseudo-random number generators are linear (or mixed congruential), which generate a sequence of integers via the relation \( x_{n+1} = \text{A} \cdot x_n + \text{C} \mod \text{M} \) where \( \text{M} \) is the modulus and \( \text{A} \) and \( \text{C} \) are positive integers called the multiplier and increment, respectively. A more popular noncongruential generator (MCG) refers to the same equation, but with \( \text{C} = 0 \). We evaluated a LCG found on many personal computers running BASIC, and a MCG, recorded in BASIC, but more commonly found on mainframe computers. The present observations summarize three aspects of these generators on an 8 MHz computer: speed, period, and uniformity. Speed of the LCG was faster (0.9 sec/sample) than that of the MCG (1.06 sec/sample). The period of the LCG was \( 2^{31} \) (16,777,216 samples; 4.2 hr) relative to \( 2^{31} \) (2,147,483,646 samples; 26 days) for the MCG. Uniformity was assessed by calculating 1000 \( x^2 \) values, each based on a \( 1000 \) values were noted, as expected under “true randomness”, for the MCG (p < .50), but not for the LCG (p < .01). These data suggest that improvements in pseudo-random number generation may qualify to be obtained with modest decrement in speed, and large curtailment in the use of any pseudo-random number generator simply because it is readily available.

**DISCREMINANT CONDITIONING OF AUTONOMIC AND SLEEPER RESPONSES (25-90 DAY) IN NEUROMUSCULAR BLOCK. B.B. DeWitt and S. DeWitt. Dep. Behavioral Science, Penn State University College of Medicine, Hershey, PA.**

Rats with bilateral sciatic tibial n. electrode cuffs are chronically paralyzed and ventilated using a continuous infusion of alpha-bungarotoxin. They have normal blood pressure, pH, R.A, and respiratory variables, anesthetic level (isoflurane), and EEG were controlled. Baseline muscle responses were recorded. They were then trained on a task under "true randomness", for the MCG (p < .50), but not for the LCG (p < .01). These results demonstrate the specificity, reversibility, and rapidity of neural inactivation produced by a cuff that can be implanted in freely behaving rats.


The purpose of this study was to test a special case for retention of a backward conditioning (BC) response in the ischemically decapitate spinal preparation. Isometric muscle tension recordings (foamicel) were used to quantify alterations in the magnitude of the flexion reflex evoked by saphenous nerve stimulation (SNS) after pairing of superficial personal n. stimulation (SPS) 50 Hz, 0.1 ms, 10 sec, with a 0.1 ms SNS. Both SC and SNS were supermaximal for activation of A-delta fibers. Experimental animals received 30 paired trials (SNS preceded SC by 0.1 ms with an IF of 3 min). Control animals received the same stimuli but in an explicitly unpaired manner. Following acquisition, all subjects received 30 additional DCS-alone trials at 5 min. intervals. Conditioned responding was monitored throughout acquisition with SC-alone "probe" trials which tested the excitability of spinal reflex circuits activated by A-delta or A-alpha plus A-delta fibers. In confirmation of previous results [L. Neurosci., 6:2411, 2001], BC treatment in this preparation resulted in significant retention. Independent t-tests indicated that significant differences existed between the overall acquisition means of paired and unpaired groups (p < 0.001). Analysis of A-alpha CS探s yielded similar results. At intensities supermaximal for A-delta fibers, CS-alone trials, following acquisition, resulted in rapid SC decrement toward control group levels. In contrast, analysis of the overall extinction means obtained with A-alpha CS-alone paired indicated evidence of extinction of the CR [J. Neurosci. 19:2, 2495, p < 0.005]. These latter findings represent the first demonstration of retention of BC effects in this preparation.

The role played by NMDA receptors in experimental epilepsy has been well documented. Here, we used intracellular recordings to study the effects of NMDA receptor antagonists on cortical neurons. Independent t-tests indicated that significant differences existed between the overall acquisition means of paired and unpaired groups (p < 0.001). Analysis of A-alpha CS探s yielded similar results. At intensities supermaximal for A-delta fibers, CS-alone trials, following acquisition, resulted in rapid SC decrement toward control group levels. In contrast, analysis of the overall extinction means obtained with A-alpha CS-alone paired indicated evidence of extinction of the CR [J. Neurosci. 19:2, 2495, p < 0.005]. These latter findings represent the first demonstration of retention of BC effects in this preparation. Thus, in the spinal cat, both forward conditioning (FC) [Behav. & Neural Biol. 43:12] and backward conditioning (BC) [Behav. & Neural Biol. 43:12] and BC procedures result in long-term reflex potentiation. However, unlike FC, which requires activation of A-delta path to produce retention of the CR, this study demonstrates that BC reflex alterations are limited to the spinal circuitry activated by A-delta fibers. These and other recent findings [L. Neurosci. 8:502] support the hypothesis that BC and FC are unique phenomena characterized by different neuronal processes. Supported by NSF grants BNS 841591 and BNS 8803945.
EPILEPSY IV

WITH POTENT ORAL ANTICONVULSANT ACTIVITY. M. Schmutz*, K. Campal slice which produces seizure-like electrographic discharges in response to kindling-like stimulations. In slices from 24-31 day-old rats, stimulus Administration Medical Center, Durham, NC Medicine, and Psychology, Duke University Medical Center and the Veterans models (high K+, low Mg+), but are consistent with their effects on kindling in vivo.

Finally, the antagonist was reapplied once the EGSs were established and stable. We conclude that, in this model, the suppression of NMDA receptor activity or the opening of the associated channels is profoundly antiepileptogenic. How­

We investigated the role of NMDA systems in kindling, the effects of systemic injection of MK-801, a novel non­

competitive antagonist of NMDA receptors, were examined in kindled rats. The results were; (i) Both the seizure stage and duration of kindling increased (50% with 0.1 mg/kg, 2.5 fold) and the number of convulsive behaviors were reduced significantly (95% with 0.1 mg/kg, 3 fold) and the number of convulsive behaviors was reduced significantly (95% with 0.1 mg/kg, 3 fold). (ii) The results suggest that the effects of MK-801 on kindled seizures, and the growth of ADs was strongly prevented. (iii) Pretreatment with reserpine did not antagonize the effects of MK-801 on AM kindled seizures. These results indicate that MK-801 has potent anticonvulsant actions on kindled seizures from both limbic and cortical foci, and that NMDA systems may play a critical role in the seizure-triggering mechanism of kindling.

SYNERGISTIC ANTICONVULSANT ACTION OF NIMODIPINE AND MK-801 IN MICE ADMINISTERED PENTYLENETERAZOL. G.P. Bolger and S.R. O'Neill. Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 2V6

Both NMDA- and AMPA preferring antagonists have been shown to possess anticonvulsant activity in mice administered the cortical convulsant pentyleneetrazol (PTZ). We have investigated the effects of i.p. administration nimodipine and the potent NMDA antagonist MK-801, either alone or in combination, on PTZ (85 mg/kg, i.p.) convulsions in male CD-1 mice. PTZ produced severe tonic convulsions and mortality was 55% (5/9), the mean onset 71 ± 3 sec; mortality 23% following full tonic-clonic seizures. Nimodipine (30 mg/kg) increased the mortality (30%–50%) from PTZ convulsions. In contrast, MK-801 (30 mg/kg) or nimodipine significantly increased (two fold) clonic convulsion onset time. MK-801 (0.1 and 0.5 mg/kg) neither altered the onset time of the convulsions nor the number of animals experiencing clonic convulsions, but did prevent death due to PTZ convulsions. Combinations of nimodipine and MK-801 at doses as low as 2 mg/kg and 0.5 mg/kg, resulted in respectively, resulted in an increased onset (three fold) and a reduction (50%) in both the number of animals experiencing clonic convulsions and the severity of the convulsions. Furthermore, only partial protection was prevented at all dose combinations of nimodipine and MK-801 investigated. These results suggest that the combination of MK-801 and nimodipine may provide a safe and beneficial adjuvant therapy for epilepsy.

(Supported by the MRC and the Faculty of Medicine, MUN).
345.9  GLYCINE POTENTIATES PHENOBARBITAL, CARBAMAZEPINE AND MK-801 IN MAXIMAL ELECTROSHOCK SEIZURE SEIZURES. D.L. Peterson, J.T. Trzeciakowski, L.M. Bonehke, and K.V. Riegel*. Dept. of Medical Pharmacology and Toxicology, Texas A & M University, College Station, Texas 77843.

The purpose of this study was to evaluate the ability of glycine to enhance the effects of clinically effective anticonvulsants in a standardized model of experimental epilepsy in rats.

Maximal electroshock seizures were induced by passing a 60 Hz, 1500 V, 0.3 sec duration current through varine soaked corneal electrodes. Seizure severity was quantified by extension/flexion ratios. Statistical significance between anticonvulsant dose-response curves with and without glycine were determined by nonlinear regression analysis.

Glycine (30 or 40 mmol/kg, p.o.) administered 1, 2, 4 or 8 hours before the seizure test had no significant effect on seizure response. However, 40 mmol/kg glycine (p.o., 4 hours before seizure test) significantly reduced the ED50 of diazepam or phenytoin. Carbamazepine (10.1 to 6.8 mg/kg, i.p.) and MK-801 (7.0 to 3.7 mg/kg, i.p.) glycine did not significantly affect the extent of duralastomic binding.

Glycine potentiation of MK-801 suggests involvement of excitatory amino acid receptors in the mechanism of section of phenobarbital and carbamazepine in maximal electroshock seizures. (Supported by NIH Grant 24566)


Previous studies have suggested that enhancement of excitatory amino acid receptor function is associated with the kindling phenomenon. We therefore quantified radiolabeled binding to kainite and NMDA receptors in brain regions of kindled rats with use of microdialytic techniques.

Adult male rats were stimulated in the angular bundle until they experienced at least 6 class 4-5 seizures of which the last 3 were consecutive. Control rats were implanted, but not stimulated. Binding experiments were carried out on slice mounted brain sections prepared either 24 h or 48 h after the last kindled seizure or 24 h after a single evoked afterdischarge. Kainite receptors were labeled with [3H]kainic acid (100 nM) in slices maintained under conditions specific for its binding to this receptor subtype. Kainite receptor binding had declined by 24-50% in stratum lucidum of hippocampal area CA1 and by 12-15% in the inner third of the dentate molecular layer compared to the non kindled state. The effects were not detected either 28 d after the last kindled seizure or 24 h after a single evoked afterdischarge. These findings suggest that depression of kainite receptor binding represents one of many transient compensatory responses that are associated with maintenance of the kindled state.

NMDA receptor binding had declined by about 10% in stratum radiatum of the hippocampal area CA1, in the stratum radiatum of the molecular layer in slices from kindled rats. None of these changes was associated with the calcium channel and supports the idea that the enhanced NMDA receptor function may, at least in part, underlie the kindling phenomenon (Mody, 1986). These reports suggested that DM sites are involved in the mediation of the anticonvulsant activity of several drugs. However, there are important species differences: in the guinea pig, 100 µM PHT and 10 µM roliprazine (at pH 7.4) increase 4 fold the affinity of DM binding without changing the Bmax. By contrast, the anticonvulsant effects are very small (pH 8.4) of NMDA receptor function in kindled rats cannot be explained by the increased expression of NMDA receptors. Rather, kindling leads to a down regulation of NMDA receptor binding in selected brain regions. (Supported by NIH grants no 16964 and NS 17771.)

345.13  DEXTROMETHORPHAN (DM) BINDING SITES: SPECIES DIFFERENCES OF ALLOSTERIC MODULATION BY DEXTROMETHORPHAN AND FLUNARIZINE. S. Clark, W.A. Wilson and A.C. Bragdon. Depts. of Pharmacology and Neurology, Duke University Medical Center, Durham, NC 27710.

The dihydropyridine (DHP) calcium channel antagonist (CVA), nimodipine (NM) has antiepileptic and anticonvulsant properties that are thought to be mediated through neuronal calcium channel DHP binding sites. The DHP binding site can be positively and negatively allosterically regulated by the benzothiazepines and phenylalkylamines/piperazines, respectively. In this investigation, we report and characterized this binding interaction at the physiologic level by examining the effects of diltiazem (0.1, a benzothiazepine) and flunarizine (FLZ, a piperazine) on the antiepileptic activity of NM. Metrazol (MTZ, 30 mg/Kg IP) is used to induce seizures in awake rats with chronically implanted EEG stimulating and recording electrodes. In the present study, we found that 10 µM (40 µM) carbetapentane (CRB) displaced [3H]DM in the low nM range in all three species, and DM, CAR and CRB protect rats and mice against maximal electroshock seizures. Besides, DM and CRB potentiate the effect of phenytoin (PHT) (Fortella and Musacchio, Brain Res., 383:629, 1986). These reports suggested that DM sites are involved in the mediation of the anticonvulsant activity of several drugs. However, there are important species differences: in the guinea pig, 100 µM PHT and 10 µM roliprazine (at pH 7.4) increase 4 fold the affinity of DM binding without changing the Bmax. By contrast, the anticonvulsant effects are very small (pH 8.4) of NMDA receptor function in kindled rats cannot be explained by the increased expression of NMDA receptors. Rather, kindling leads to a down regulation of NMDA receptor binding in selected brain regions. (Supported by NIH grants DA-60213, MH-29591, MH-17785 and NS-23926.)


Seizure activity can be studied in vitro using brain slices containing hippocampal (HC) and entorhinal cortex (EC) bathed in ACSF containing no added Mg++ (0 Mg++), seizures can be studied for long periods if a Low Ca++ ECSF until stable seizure activity occurred, and then the carbamazepine was added. Carbamazepine, at clinically relevant concentrations (2-12 µM) suppressed spontaneous seizures, increased the threshold required to trigger seizures, and shortened the duration of the seizures. (Supported by NIH grants NS 17771 and the Veterans Administration.)
345.15

Spontaneous epileptiform bursts were recorded in the CA1 subfield of rat hippocampal slices perfused with Mg-free medium. These epileptiform events occurred at a frequency of 0.3-0.05Hz, each lasting 50-500ms. Addition of 2mM VPA evoked a decrease of the frequency of occurrence of the epileptiform bursts and a 100-200% increase in duration of each event. In addition prolonged shifts (duration 8-20s) could appear at this stage. These changes were eventually followed by the complete disappearance of paroxysmal activity. A full recovery of the low Mg bursts was observed following VPA wash. Lower doses of VPA did not affect the occurrence or the shape of the low Mg epileptiform bursts. Furthermore in the presence of bicuculline, VPA (2mM) failed to reproduce the effects described above. These results demonstrate an action of VPA upon the low Mg epileptiform activity although the doses required for blocking these bursts appear larger than those used in other "in vitro" models of epilepsy.

345.16

Gabapentin (1-aminomethyl)cycloloxoximeacetic acid is a new anticonvulsant currently in clinical trials. Gabapentin has a novel profile of activity against experimental seizures in animals, so electrophysiological experiments were done to investigate cellular mechanisms.

In intracellular recordings from cultured mouse spinal cord neurons, gabapentin (175 µM) had no effect on sustained repetitive action potentials, spontaneous synaptic activity, or iontophoretic GABA or glutamate responses. In extracellular recordings from hippocampal slices, gabapentin (100 µM) had no effect on induction of long-term potentiation. In extracellular recordings from the hippocampal CA1 area of rats in vivo, gabapentin (2.1 mg/kg IP) caused a dose-dependent decrease in inhibition measured by paired-pulse stimulation. The cellular mechanism of gabapentin's action on paired-pulse inhibition is not known. These results indicate that at relevant concentrations gabapentin does not block sodium-dependent action potentials like phenytoin, carbamazepine, or valproate. Gabapentin does not alter long-term potentiation like antagonists of the NMDA glutamate receptor subtype, but has effects similar to phenytoin on paired-pulse inhibition in vivo.

345.17

Kindling was used as a model to examine the effect of aspartame on the seizure threshold. Male, Fischer rats were implanted unilaterally with bipolar electrodes to the right hippocampal and cortex and were stimulated until 3 consecutive stage 5 convulsions were reached. If aspartame (1 g/kg, p.o.) was given 2 hrs prior to the first stimulation, which was given hourly, followed by a second dose 6 hrs later, the rate of kindling was not affected. If rats were dosed for 14 days (1 g/kg twice daily) and stimulations given hourly on the next day, aspartame had no effect. Aspartame also had no effect on the rate of kindling if rats were dosed twice daily (1 g/kg) and stimulations given hourly on the next day. Aspartame had no effect. Aspartame also had no effect on the rate of kindling if rats were dosed twice daily (1 g/kg) and stimulations given hourly on the next day. Aspartame had no effect. Aspartame also had no effect on the shape of the low Mg epileptiform bursts. Furthermore in the presence of bicuculline, VPA (2mM) failed to reproduce the effects described above. These results demonstrate an action of VPA upon the low Mg epileptiform activity although the doses required for blocking these bursts appear larger than those used in other "in vitro" models of epilepsy.

345.18

PR 934-423 (+)-2-amino-5-(1-methyl-1,2-diphenylethyl)acetamide·HCl has been shown to protect rodents against maximal electroshock seizures (MES). The favorable oral efficacy/safety ratio and low toxicity led to Phase I of clinical testing. Evaluation of the isomers of PR 934-423 revealed the following results (oral doses in mg/kg) (results with the racemate are presented for comparison): ED50 MES in mice, rats: PR1032-646 (+) 76, 33; PR1032-646 (-) 45, 20; & PR 934-423 (+) 32, 15.5. Onset of MES protection at the ED50 was 30 min (all compounds) and the duration was 4 hrs for the isomers and 3 hrs for the racemate in mice while in rats these time courses were 30 min and 8 hrs. Values for neurotoxicity (TDSO-inverted screen) and safety (LD50) in mice were: 647 and 31,000 for PR 1032-646 (+); 598 and 723 for PR 1032-646 (-); and 396 and 877 for PR 934-423 (+). The resulting therapeutic indices (TDSO/ED50) and safety margins (LD50/ED50) were: 8.5 and 912. 3.12 and 16.1, and 7.6 and 16.9 for the (+), (-), and racemate respectively. Subchronic dosing of the ED98 to mice resulted in a twofold increase in the ED50 for protection against MES. The findings indicate that in mice the (+) isomer is more potent, while in both species the (-) isomer is weaker. In addition, tolerance to MES protection occurs in mice with the racemate, as well as with the two isomers.
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early and are not directly correlated with molecular layer development over the first 3 weeks of life, establishment of adult segment number and transverse dendritic spread occur very early and are not directly correlated with molecular layer maturation.

In contrast, both the number of segments (346.3) and the length of segments (346.5) are significantly reduced in immature astrocytes. In the absence of Ca++, neuron binding was substantially reduced to mature but, not to immature astrocytes. These results indicate that the capacity of astrocytes to support neurite outgrowth and neuronal adhesion is reduced in immature astrocytes.

In this study we have examined neurite outgrowth and neuronal adhesion on astrocytes and neuronal adhesion in vitro. Astrocytes were purified from newborn rat forebrain to 95% purity by differential adhesion and allowed to mature in vitro (serum 64/64, media [346.5]). Immature astrocytes were plated onto poly-L-lysine coated cover slips at a density of 60,000 cells to form a monolayer and maintained 24 hours before the addition of neurons. Neurons were isolated from embryonic day 16 (E16) and seeded at a density of 5,000 neurons/cover slip to compare neurite outgrowth. Neurons were labeled with benzyl cyan 15, 25, and 40 hours after plating and their length determined.

The rate of neurite outgrowth appeared linear on both immature and mature astrocytes. However, neurites were consistently 30-35% longer on mature astrocytes. For short term adhesion experiments, neurons were plated at a density of 5,000 neurons/well in either the presence or absence of Ca++ (2 mM EDTA). In the absence of Ca++, the number of bound cells was reduced by 50% compared to neurons in the presence of Ca++. These results indicate that the capacity of astrocytes to support neurite outgrowth and neuronal adhesion is reduced in immature astrocytes.

Supported by APA grant R8-20; NSF grant NSF.2597-07

PERIPHERAL OUTGROWTH OF SYMPATHTIC PREGANGLIONIC NEURONS FROM AN HETEROGENEOUS THORACIC NEURAL TUBE TRANSPLANT.

The thoracic nerve roots were derived from thoracic neural tubes transplanted to the brachial region of experimental (Thor-Br) chick embryos, however, a morphologically normal brachial plexus, grafts were observed. This occurs despite the fact that the graft develops structural characteristics of an innervating thoracic (Tn) and a sympathetic column of Tn (Te). The latter (Te) is absent within the brachial region of control (BrBr) embryos. The results demonstrated that the pattern of ectopic CT fibre outgrowth from the brachial region of Thor-Br embryos replicated that of in situ CT fibres in the thoracic region. In Thor-Br embryos, CT fibre courses ventrolaterally within thoracic NT grafts, entered ventral motor roots and then, diverged from the peripheral nerve to enter the brachial sympathetic trunk. This pattern was observed from day 6 to day 18.

Currently, the fate of ectopic CT fibres within the periphery is under investigation. (Supported by MDMA and NSERC)

The factors that control the development of the dopaminergic axons from the substantia nigra to the striatum remain unknown. We have used a transgenic mouse line that expresses green fluorescent protein in migrating neurons and have been suggested to guide axons during development. In the rat at ages E12, E13, E14, and E16 we analyzed the paths of the nigrostriatal axons and processes of radial glia using a polyclonal anti-serum against tyrosine hydroxylase (TH) and a monoclonal antibody against visinin, respectively. At E16 TH-like material was not detected in the developing rat brain. However, visinin-like material was widely distributed. At E13 TH-like cells, which later develop into the neurons of the substantia nigra and ventral tegmental nucleus, were clearly apparent at the mesencephalic flexure with projections toward the developing striatum. The TH-like processes of TH-like axons projecting to the developing striatum were clearly apparent. At E16 TH-like axons emanating from the mesencephalon did not parallel visinin-like processes. However, more caudally the path of the developing nigrostriatal TH-like axons was similar, through not entirely overlapping with a group of visinin-like processes.


In addition to its function as a neurotransmitter, y-aminobutyric acid (GABA) influences a variety of processes in the developing central nervous system (Rubin, O.A., and Schauf, A. eds. Neurosci. 32, 1986). Little is known about the distribution and function of GABA in the developing peripheral nervous system. We have investigated the distribution and time course of transient GABA immunoreactivity in chick embryos from 2 to 16 days of incubation (E2-E16). GABA immunoreactivity occurs transiently in motor portions of all cranial nerves (between E4-E10), in restricted parts of the peripheral ganglia (E4-E5), including its central and peripheral processes, and in some, possibly, transient projections from the brain to the spinal cord. In addition, we observed differential onset of GABA immunoreactivity in the oculomotor (E4) and facial (E6), including its central and peripheral processes, and in some, possibly, transient projections from the brain to the spinal cord. The present studies were undertaken to determine the fine structural location of this transient GABA activity. Sprague-Dawley rat pups of 8-12 postnatal days of age were perfused with aldehydes and vibratome sections were processed for GABA histochemistry. Some animals received unilateral lesions of the dorsolateral thalamus 36-48 hr prior to sacrifice. GABA stained sections containing the lateral or medial geniculate body or primary or auditory or cortical areas were processed for electron microscopy (Seres et al., Dev Brain Res. 16, 1983). GABA histochemical reaction product was found associated with the granular endoplasmatic reticulum and nucleus of virtually all large projection type neurons of the lateral and medial geniculate nuclei. Glial cells were stained. In visual and auditory cortices GABA reaction product was found associated with the plasma membrane of axon terminals as well as with some of the terminals that received thalamic lesions prior to sacrifice. GABA reaction product could be found associated with the plasmalemma of degenerating axon terminals in both visual and auditory cortices. The present results are consistent with previous data indicating that GABA is synthesized transiently by thalamocortical projection neurons during the early postnatal period of development.

Supported by NSF grants BNS 87-08515 and BNS 86-15579 and NIH grants NS-25674 and NS-15066.

346.11 CYTOSKELETAL INVOLVEMENT IN RESEALING OF NEURONAL MEMBRANES AFTER INJURY. A.-F. Xie and J.N. Barrett. (ZPON: Di Puro). Dep. of Physiology & Biophysics, Univ. of Miami School of Medicine, Miami, FL 33101.

Axons of cultured rat septal neurons were transected by a laser and examined at 0, 5, 10, and 30 min. The transected neurons take up Lucifer Yellow and FITC-conjugated dextran (18 kDa and 70 kDa) before, but not after, membrane reseal. Using dye backfilling, we found that 75% of the axons resealed within 20 min in control medium containing 2 mM Ca, 0.1 mM GABA, and 100 µM L-arginine. Neurons were incubated for 12 h in the presence of L-arginine followed by resealing with 2 mM Ca, 0.1 mM GABA, and 100 µM L-arginine. Resealed axons were identified by their characteristic change in neuronal diameter and the presence of a characteristic fine structure. The resealed axons were then examined using a combination of electron microscopy and immunohistochemistry. The results suggest that the normal mechanism for membrane resealing is Ca-dependent and involves cytoskeletal elements and calcium. Surprisingly, resealing in low ionic strength solutions (5 mM in isotonic sorbitol or sucrose) lacking divalent cations, indicating that certain essential resealing ionic components may also be achieved by a Ca-independent pathway. Supported by NIH grant NS 12207.


The tangential nucleus (TN) is a primary vestibular nucleus which contains the principal cells (PC) that migrate at 6-8 days and differentiate between 6 and 8 days. Although the primary vestibular afferents to the medulla in fascicles at 3 days, these fascicles delay forming synapses on PC until 7 days. In fact, the very first synapses are found primarily on the most rostral of the primitive epithelial cells (PEC; PC precursors) by longitudinal fibers (LF) of unknown origins. Therefore, the present object was to determine what structural interactions occur in the TN between 2 and 5 days. Before the 7-th nerve fibers grow in, the presumptive TN contains processes of PEC separated by empty spaces, or "channels." The LF appear within the channels at about the time of entrance of the 7-th fibers. From 2 to 4 days, no synapses are apparent in the TN. However, attachment plaques and coated inclusions are present on the membranes of the components of the TN, including the primary vestibular afferents, PEC, and LF. Thus, attachment plaques and coated inclusions are present on the synaptogenesis in the TN. This data will provide a basis to evaluate experimental studies on TN development in the absence of the primary vestibular afferents. Supported by NIH grant RO1 NS31808.


We have developed a low density hypothalamic cell culture system. Media containing serum was dissected from 2-5 day old rats by 45% dissociated (10 U/ml papain) and plated directly on glass coverslips coated with antibodies to Thy 1, a CNS surface protein. This substrate allows excellent survival and growth of virtually all large projection type neurons of the lateral or medial geniculate nuclei. Gabaergic neurons were unstained. In visual and auditory cortices AChE reaction product was found associated with the plasma membrane of axon terminals as well as with some of the terminals that received thalamic lesions prior to sacrifice. AChE reaction product could be found associated with the plasma membrane of degenerating axon terminals in both visual and auditory cortices. The present results are consistent with previous data indicating that AChE is synthesized transiently by thalamocortical projection neurons during the early postnatal period of development.

Supported by NSF grants BNS 87-08515 and BNS 86-15579 and NIH grants NS-25674 and NS-15066.


Previous work from this laboratory has demonstrated transient patterns of acetylcholinesterase (AChE) histochemical staining in layer IV of primary sensory cortical regions of developing rats (Robinson, Neurosci. Lett. 75/259, 1987). The laminar and areal distributions of transiently expressed AChE activity correspond to the terminal fields of specific sensory thalamocortical projections, suggesting that transient AChE may serve as a marker for these thalamocortical neurons. The present studies were undertaken to determine the fine structural location of this AChE activity.

Sprague-Dawley rat pups of 8-12 postnatal days of age were perfused with aldehydes and vibratome sections were processed for AChE histochemistry. Some animals received unilateral lesions of the dorsal thalamus 36-48 hr prior to sacrifice. AChE stained sections containing the lateral or medial geniculate body or primary visual or auditory cortex were processed for electron microscopy (Seres et al., Dev Brain Res. 16, 1983). AChE histochemical reaction product was found associated with the granular endoplasmatic reticulum and nucleus of virtually all large projection type neurons of the lateral or medial geniculate nuclei. Gabaergic neurons were unstained. In visual and auditory cortices AChE reaction product was found associated with the plasma membrane of axon terminals as well as with some of the terminals that received thalamic lesions prior to sacrifice. AChE reaction product could be found associated with the plasma membrane of degenerating axon terminals in both visual and auditory cortices. The present results are consistent with previous data indicating that AChE is synthesized transiently by thalamocortical projection neurons during the early postnatal period of development.

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B. Snyder*, M. A. Murray, and J. Palka. Dep. of Zoology, Univ. of Hawaii, Honolulu, HI 96822.

Unlike most neurons in primary culture, which require conditioning factors for outgrowth, the secretory neurons of the crab (Carcinosoma cancellatum) and lobster (Homarus americanus) X-organ show immediate, vigorous outgrowth in simple, defined media (e.g. crab saline + glucose, gentamicin, pH 7.6, 25°C) when isolated on a variety of substrates (routinely, Primaria within 18 h the morphology is established: small cells (<25 μm), which include those showing RPHC immunoreactivity, have a sparsely branched major neurite extending >100 μm from the axonal stump; some larger cells form similar, but less produce a broad lamellipodium, often larger in area than the soma. These show immunoreactivity to anti-GH, anti-MH or anti-D sera. Growth of veiling cells ceases by 10d, while branching cells continue longer. Additional growth and altered form result from various manipulations such as brief exposure to elevated [K+], or co-culture with a sinus gland. We suggest that these cells can show immediate outgrowth by utilizing part of the secretory machinery, exocytosis without membrane reuptake, for addition of membrane. In support: a) EM's show microtubules and secretory granules in filopodia; b) growth cones and presumed Golgi are particularly immunoreactive; c) hormonal peptides are released to the media; d) cells exhibit Ca currents (Meyers et al., this vol. 4) growth, secretion and Ca currents are blocked by Cd. We thank H. Schooneveld and R. Keller for antisera. Supported by NSF BNS84-04459 and NIH NS 15453 to LC.


The mechanism by which orderly axonal projections and the corresponding synaptic connections are formed during development remains an important and largely unsolved problem in neurobiology. It may be possible to bring genetic and molecular biology techniques to bear on this problem by examining the genetic control of axonal growth in Drosophila. We show here that axonal projections in Drosophila are sexually differentiated and that genes involved in sex determination control the anatomy of these axons. Both males and females possess gustatory receptors on their legs and in females the axonal arborization from these receptors is exclusively unilateral. Males possess significantly more gustatory receptors, and their axonal arborization is usually bilateral. In diplo-X "males" mutant for the gene tra or tra, the gustatory system is transformed toward the male phenotype both in number of receptors and in afferent projections. In order to determine the locus of this effect we examined gynandromorphs and found that the sex of the sensory neuron and apparently not the central nervous system, controls the behavior of the axons. Supported by an NIH Javits Neuroscience Investigator Award to RKM.

SPECIALLY RESTRICTED BINDING OF MONOCLONAL ANTIBODIES AGAINST WINGS OF DROSOPHILA MELANOCERUS. P. B. Snyder*, R. A. Graf, S. Gran*, B. Haylett, and J. Cooke. Békésy Laboratory of Neurobiology and Department of Zoology, University of Hawaii, Honolulu, HI 96822.

Because of the strong evidence that significant guidance information is provided to pioneer sensory axons in the Drosophila wing by the epithelial substrate, we have generated monoclonal antibodies against wings during the amnioserosa period. 2G2 and 3C11 stain extracellular material located throughout the wing but concentrated along the veins. This distribution is similar to that of a vein-associated antigen we have described previously (Murray, M. A. & Paulus, J. Abstr. Soc. Neurosci., 13, 1143, 1987.). 2G2 shows staining exclusively along the third vein (one of two axial tracts in the wing blade). This pattern is reminiscent of the binding distribution in Drosophila wings of INO, a monoclonal antibody that inhibits neurite outgrowth of cultured mouse neurons (Matthew, N. D. & Patterson, P. H. Cold Spring Harbor Symp. Quant. Biol., 48, 625-631, 1983; Blair, S. S. & Paulus, J. Abstr. Soc. Neurosci., 13, 6, 1987.). Hence, we currently have a collection of monoclonal antibodies, presumably directed against extracellular matrix components, which show overlapping but distinct distributions. We plan to analyze their binding in other Drosophila tissues and biochemically characterize the corresponding antigens. We also hope to be able to address the question of their possible role in axon growth.


The giant fiber pathway in Drosophila mediates a quick escape response. The giant fiber synapses with the TM motoneuron (TTM) innervating the TM, or jump muscle. The giant fiber also innervates a postsynaptic neuron which then synapses upon the motoneurons for the DLM wing depressor muscle (Wyman et al. in: Neural Mechanisms of Startle Behavior, R.C. Eaton, ed. Plenum 1984). A mutant was identified on the 3rd chromosome in which the pathway to the DLM is electrophysiologically normal, but the response of the TM is aberrant, having a delayed latency much like the X-chromosome mutant bendix. Cobalt fills of the giant fiber through the antennal nerve reveal that the giant fiber is morphologically normal. Horseradish peroxidase backfills of the TTM's from the TM indicate the TTM is connected to the jump muscle and not apparent normal. Physiological experiments are under way to pinpoint the lesion in the pathway from the giant fiber to the jump muscle. The mutation has been mapped to the proximal left portion of the third chromosome.
Each side of every trunk segment in the zebrafish is innervated by 3 identified motorneurons that extend axons out of the spinal cord in a stereotyped temporal and spatial sequence. As the growth cones of these cells follow a common pathway to a region where they pause before selecting cell-specific pathways that lead to motor nuclei in the territory in which they are tested, CaP, the first motorneuron to extend a growth cone, had a unique ability to establish the common pathway. Ablation of CaP in the chick embryo does not affect the ability of the growth cones of the other identified motorneurons, MIP and RoP, to leave the spinal cord and extend along the common pathway. We also tested whether interactions among motorneurons were required for cell-specific pathway choice. Following CaP ablation, the MIP and RoP growth cones entered the motorcell-specific pathways. These results suggest that CaP is not necessary to establish the common pathway, and that interactions with CaP are not required for MIP and RoP initially to select their normal, cell-specific pathways. We are currently examining whether interactions among motor growth cones may affect later pathway choices. Supported by the NIH, NSF, Chicago Community Trust and a Patricia Roberts Harris fellowship.

PEPTIDOLIGOSMAL (PNA) BINDS TO TISSUES THAT ACT AS BARRIERS TO AXON ADVANCE IN THE CHICK EMBRYO. R. A. Olszowy & K. Tsony. Neurosciences Program & Biology Department, University of Miami, Coral Gables, FL 33124. Several tissues in the developing chick embryo can be viewed as barriers to axon advance. These include the pelvic girdle precursor, the posterior notochordal mesenchyme (ventromedial somite surrounding the notochord). Surgical deletion studies have shown that nerve paths expand spatially when the pelvic girdle or sclerotome are deleted and that motor nerve guidance depends on interactions with notochordal mesenchyme (Tosney & Landmesser, J. Neurosci. 4: 2518; Tosney, Dev. Biol. in press). The posterior sclerotome is resistant to axon invasion and has been shown to differentially label PNA (Stain et al., 1987). To determine if the binding of this lectin is typical of barrier tissues, we studied PNA binding in chick embryos in relation to the timing of axon outgrowth.

Stage 18-25 embryos were processed according to Stain et al. Frozen sections (10 µm) were stained with FITC-PNA. We found definitive binding of PNA to the pelvic girdle precursor, posterior sclerotome, and to the notochordal mesenchyme of both posterior and anterior sclerotome. In anterior half segments, PNA binding was specifically limited to the perinotochordal mesenchyme and did not include the spinal nerve pathway. PNA binding in the posterior region was detected more generally, up to the myotome and to the limb base. PNA binding to the pelvic girdle precursor was detected at the earliest growth cone invasion the pelvic region just medial to it. PNA did not bind to nerve paths involving the plexus region and the histogenes through the girdle which normally transmit axons to the limb. Thus, a PNA binding epitope is common to several barrier tissues and may be involved in constraining the patterns of axon outgrowth. Supported by NIH NS23108.

DISAPPEARANCE AND REAPPEARANCE OF FIBRONECTIN ALONG AXONAL PATHWAYS OF THE DEVELOPING PERIPHERAL NERVOUS SYSTEM OF THE CHICK. J.W. Hig and T.P. Vase. Dep. of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15266. A molecule that has been shown to modulate morphogenetic movements during gastrulation and mediate the 3-dimensional outgrowth and migration of neural crest cells is the glycoprotein fibronectin. Although fibronectin has also been shown to promote neurite growth of dorsal root ganglia in culture, it is less clear. As we demonstrated in our previous note, quail cells were observed in the dorsal root ganglia, the sympathetic ganglia, and in the ventral roots of spinal segments affected by the grafts. In addition, quail cells were seen in the spinal nerves and plexus in peripheral nerves innervating the wing. Quail cells were distributed along the entire length of the nerve pathway, with increased concentrations of quail cells present in the plexus region and at nerve branch points. Quail cells were not seen in advance of the growing nerves at any of the stages examined. Quail-derived Schwann cells were distributed in the peripheral nerves and axon segments affected by the grafts. The pattern closely matches the pattern of innervation established by motor axons emerging from the same segmental level of the spinal cord. These results suggest that Schwann cells accompany emerging nerve fibers from the same segmental level and use these fibers as substrates to guide their migration into the periphery. Supported by NIH grant NS 25340 to MH. EMG is supported by PHS GM 7839.

MESENCHYMAL CELL DEATH DELINATES AXON PATHWAYS IN THE HINDLIMB AND DOES SO INDEPENDENTLY OF NEURAL INTERACTION. S. Schreger, J. A. Pokrzywinski* and K. W. Tsony. Laboratory of Developmental Biology, The University of Michigan, Ann Arbor, MI 48109. We wished to know whether the mesenchyme cell death seen near growth cones in the hindlimb delineates axon pathways and, if so, whether the death was "murder" (an interaction with growth cones) or "suicide" (independent of motor axon interactions and therefore characteristic of whatever processes generate the paths). We unilaterally deleted the lumbar sacral neural tube and reconstituted the patterns of neurite outgrowth and mesenchyme cell death during the stage when neurites first colonize the thigh. In the control limbs, axonal pathways coincided with sites of cell death. Dying cells were abundant where axons ramified extensively, such as plexus regions and at foci within the muscle masses that correspond to regions where muscle nerves will form. In contrast, dying cells were not seen in adjacent non-pathway regions, such as posterior sclerotome or dorsal and ventral root regions of the plexus in which axons extend only posteriorly. In the experimental limbs, neurite outgrowth was reduced to less than one-twelfth of normal (a few neurites were visible with electron microscopy) or to less than one-third of normal, extended less far distally and, in half the cases, motor innervation was completely abolished. Despite the extensive reduction in neurite outgrowth, the distribution of cell death was indistinguishable from the control side. Furthermore, it did not differ significantly in abundance. We conclude that mesenchyme cell death delineates axon pathways remarkably well and does so without an interaction with growth cones. It is an independent characteristic of axonal pathways and may in some way help to guide axons. Supported by NIH grant NS 21308.
RESPONSE OF REGENERATING GOLDFISH RETINAL AXONS TO TECTAL MEMBRANES OF ADULT FISH AND EMBRYONIC CHICK
L. Valim¨et(1), C.O. Schaper, Friedrich-Miescher-Laboratory, der Max-Planck-Gesellschaft, 740 T€ubingen FRG

Retinal axons from embryonic chick (E6) in vivo respond to position specific properties of membranes of embryonic tecta (E9 to E13), as shown by Walter et. al.1987.

Employing their assay they pursued regenerating retinal axons from adult goldfish recognize differences between rostral and caudal membranes 1. of adult fish tecta and 2. of embryonic chick tecta. Cell membranes were prepared from either rostral or caudal tectum and arranged in alternating narrow stripes. Fish axon growth over these stripes demonstrated that fish axons have preferences for particular types of membranes.

1. Adult fish axons, having been grown on the rostral tectum, grew over all 6 regions, but they quickly joined one of the three bundles. By E12, the new ganglion neurons, pseudounipolar at this stage, have reached their target area where their axons branch out heavily.

These results suggest that axons of spinal interneurons may utilize specific "short-range" topographic cues for maintaining their longitudinal trajectories. However, in the absence of these cues (e.g., after 180° rotation), axons can continue to extend in a "foreign" environment over relatively long distances. Supported by NSF 8707290.

130 KD LEECH PROTEINS ISOLATED WITH MAbs GENERATED AGAINST N-GLYCANASE TREATED IMMUNOBLOT BANDS. L.S. Thogersen and B. Zipser
Dept. Physiology, Michigan State University, East Lansing, MI 48824.

Previously, a group of cell type specific 130 kD glycoproteins has been characterized in leech nervous tissue using monoclonal antibodies (mAbs). The antibodies specifically recognize sets and subsets of sensory afferents, axon tract glia or connective tissue. They were generated against fixed CNS tissue of the leech Hae mopis marmorata using monoclonal antibodies (mAbs). In the leech, the mAb Laz6-297 binds to a 130 kD surface glycoprotein expressed by macroglial cells associated with the central neuropil, interganglionic connectives, and the ganglionic roots.

We are studying the differentiation of macroglial cells in the developing nervous system of the leech Haemopis marmorata. The results of earlier experiments have revealed that the adult, the mAb Laz6-297 binds to a 130 kD surface glycoprotein expressed by macroglial cells associated with the central neuropil, interganglionic connectives, and the ganglionic roots. Before ganglia and axon tracts are formed (5 days of development at 20°C), a novel cell line of cells is detected by Laz6-297 in the midline of the embryo. This novel cell line consists of a few cell bodies confined to the region of the third and fourth thoracic ganglia, and a short process extends anteriorly and laterally from the third thoracic ganglion. This cell line, which is consistent with the boundary between the right and left halves of the third thoracic ganglion, is detected in an arrangement similar to the more numerous midline cells detected in the leech Hirudo medicinalis by the mAb Lan0-2 (McClellan-McCulloch and Zipser, Soc. Neurosci. Abstr. 13:1211, 1987). During axon tract formation, Laz6-297 stains macroglial cells of the connectives, neurup, commissures, and roots in an anterior-posterior temporal gradient. For example, at 10 days of development (20°C) Laz6-297 stains the anterior half of the nerve cord, but by day 11 more than two-thirds of the nerve cord is labeled. This staining pattern reveals three processes exiting each side of the posterior ganglia and the apparent fusion of the two anterior processes in the anterior ganglia.

The early labeling by Laz6-297 allows us to characterize the ontogeny of glial cells and the nature of glial-neuronal interactions during development. Of particular interest to us are the interactions involving different 130 kD surface glycoproteins which distinguish glia, neurons, and connective tissue during neurogenesis.

We are studying the formation of central and peripheral axon tracts in locust embryos. In this study, we have identified the earliest processes identified in the future connective that belong to the transient bipolar cells which, because of their morphology and time of appearance, were suggested to play a role in the establishment of the axonal pathways. Our data on the special characteristics of bipolar cells in head and tail ganglia further support this idea: (1) Bipolar cells in the head ganglia extend their processes rostrally to the edge of the germinal plate and persist late into development, until the tract between the head and supraesophageal ganglion is formed. Their extended survival is in contrast to the gradual loss of bipolar cells observed in nearby ganglia. The bipolar cell pattern of the head may persist to help form the axon tract. (2) In tail ganglia, bipolar cells deviate from their usual (upright) course and project caudally, forming a terminal loop. Thus, bipolar cells may delimit the posterior extent of the connective. Peripherally, early stained processes belong to sensory afferents which may pioneer these peripheral tracts. Therefore, processes postulated to pioneer central and peripheral axon tracts share the earliest cytoskeletal epitopes detected in primordial ganglia and peripheral neurons. Ten percent of the central neurons also express Laz1-1 reactive cytoplasmic epitopes as they differentiate. These Laz1-1 reactive processes are only partially Triton-X100 extractable under conditions which do not extract Lam8 reactive cytoskeletal epitopes. The staining of large cell bodies tends to fade with detergent extraction while the staining of small cell bodies remains strong. We are trying to determine which cytoskeletal associated proteins Laz1-1 may recognize.

INTERACTIONS BETWEEN NEUROTRANSMITTERS II


We have previously shown that NA, by acting at α1-adrenergic receptor, potentiates the accumulation of cAMP elicited by VIP in mouse cerebral cortical slices (J. Neurosci. 5, 356-368, 1985). This α1-receptor-mediated action of NA is antagonized in a concentration-dependent manner (EC50 = 3 μM) by indomethacin and dicyclofenac, two inhibitors of cyclooxygenase, and by mepacrine and p-bromo-phenacylbromide, two inhibitors of phospholipase A2 (Nature 367, 637-640, 1987). These observations indicate that prostaglandins may potentiate the accumulation of cAMP elicited by VIP. Among various prostanoids tested, only PGE2 and PGF2α mimic the action of NA. Inhibition of diacylglycerol lipase (by RH C 80267) and of protein kinase C (by H-7) are without effect on the potentiation effect of NA, thus discounting a role of phospholipase C activation in this α1-adrenergic mechanism. HVD, via H1 receptors, and AD (at μM concentrations) also potentiate the increase in cAMP elicited by VIP in an indomethacin- and dicyclofenac-sensitive manner, hence indicating that prostanoids are also involved in this synergistic interaction. These results indicate a role for prostanoids in the amplification of the action of a peptide in the mammalian CNS.

BEHENI WHITLEY C4-BINDING SITES ARE S-ALKYLGLUTATHIONE BINDING SITES. A. M. Goffinet* Positron Tomography Laboratory, Univ. Louvain Med. Sch. Louvain-la-Neuve, Belgium.

Recent studies (eg Goffinet & Nguyen, Rur P Pharmcol 140: 343) have demonstrated the presence of leukotriene C4 (LTC4) binding sites in brain membranes and tissue sections and have been taken as evidence that LTC4 may serve some modulatory function in the central nervous system (CNS). So far, however, the nature and function of putative brain LTC4 receptors remain controversial.

In this work, we show that leukotriene C4 binding to brain membranes is readily displaced by S-alkylglutathiones derivatives, with the affinity of the test compound increasing as the alkyl chain length increases. S-methyl- and ethyl glutathione are equipotent with glutathione. S-butylyl-, hexyl-, octyl-, nonyl- and decylglutathione show increasing activities. S-decylglutathione is almost as potent as leukotriene C4 itself.

These data strongly suggest that brain LTC4 binding sites are actually membrane-bound, glutathione-binding proteins such as glyoxalases I and II and/or, more likely, a microsomal form of glutathione transferase.

MONOCO NAL ANTIBODIES AGAINST CONJUGATED NEUROTRANSMITTERS AND THEIR IMMUNOCYTOCHEMICAL APPLICATIONS. J.J. Chanawji, J. Van De Moortele, M. Souna, N. Mins, M.C. Roherier and M. Seurat, ONCOS, 53077, Bordeaux, France. For the simultaneous detection of chemical-defined neuronal pathways in rat central nervous system (CNS), we use poly and monoclonal antibodies directed against conjugated small-sized neurotransmitter (NT) molecules. The development of such antibodies required the following methodological aspects: (i) the mouse polyclonal immune response directed against each conjugated NT was carefully monitored using a modified ELISA method; (ii) according to the conjugated hapten, we used for the hybridization of the following conjugate proteins: (iii) as soon as hybridomas producing specific antibodies against the conjugated NT could be discriminated from those producing antibodies against the coupling agent-carrier residue, affinity and specificity studies were carried out using conjugated NT closely related. We have recently raised monoclonal antibodies against: conjugated glutamate (10μM), conjugated GABA (5μM), conjugated dopamine (10μM), conjugated norepinephrine (10μM), and conjugated serotonin (2x10−6M). Thanks to our polyclonal and monoclonal antibodies and the immunocytochemical procedures, we have investigated the studies of the anatomical relationships between the various aminergic, cholinergic and monoaminergic systems. The simultaneous localization of separate antisera and monoclonal anti–glutamate antibody on the glutaredylex-fixed sections from rat brain enables us to specifically visualize these two NT. Many combinations will further facilitate the understanding of the chemical relationships existing in the neuronal circuitry.
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The action of met-Enk on ganglion cells, as well as an indirect, synaptic inhibitory action presynaptic to GC and amacrine cells. These targets may receive additional synaptic causes a net inhibition of GC. When GABA synapses are blocked, there is increased broadening of ganglion cell receptive-field center and reduction of surround spontaneous activity evoked by K+.


We examined the ultrastructural localization of the adrenergic synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT) and gamma-amino butyric acid (GABA) in the more caudal, cardiovascular, portions of the medial nuclei of the solitary tracts (m-NTS). Peroxidase reaction for GABA and immunohistochemical labeling for PNMT were principally, but not exclusively, found in different populations of perikarya, dendrites and terminals. The GABAergic neurons received synaptic input from other unlabeled terminals and from terminals containing either GABA or PNMT-like immunoreactivities. Conversely, the PNMT-labeled neurons received synaptic input from unlabeled terminals and from terminals labeled for either PNMT or GABA. In some cases, the same unlabeled dendrites received synaptic input from terminals that were immunoreactive for PNMT and GABA. These results indicate that in the m-NTS adrenergic neurons (1) modulate and are modulated by other adrenergic and GABAergic neurons and (2) share common neuronal targets with GABAergic neurons. (Supported by grants MH00078 and HL19874).

873.5


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873.6


The ultrastructural distribution of CHAT- and GAD-immunoreactivity (IR) in the rat interpeduncular nucleus (IPN) was examined employing the sequential double antigen procedure of Levey et al. (1986). CHAT-IR was demonstrated with diaminobenzidine and benzidine dihydrochloride. Each chromogen exhibited a distinct ultrastructural appearance. The central and intermediate subnuclei and the rostral half of the rostral subnucleus contained predominantly CHAT-IR and GAD-IR (mixed) neurons. The caudal subnucleus contained CHAT-IR dendrites and GAD-IR somata. Symmetrical contacts between CHAT-positive terminals and GAD-positive dendrites were observed occasionally in the rostral subnucleus.

873.7

RELEASE OF NEUROPEPTIDES FROM SYMPATHETIC NERVE TERMINALS IN VIVO. K. Ho, J. Ingersoll, H. Zhang, P.J. Christoffel*, T.J. McDonald*, and M.A. Cook. Departments of Pharmacology and Toxicology, and Medicine, University of Western Ontario, London, Ontario, Canada N6A 5C1.

A preparation of enteric sympahtochromes obtained from guinea-pig myenteric plexus was used to characterize the release of substance P (SP), gastrin-releasing peptide (GRP) and bombesin in the presence of naloxone. The potency orders obtained were: For α-NKLI, GRP>GRF>Bomb; for SPl, GRF>Bomb; for LEI1, GRF>Bomb; and for Galli, Bomb>GRF. These distinct profiles are consistent with heterogeneity of GRP receptors on enteric nerve endings. It is concluded that enteric sympathetic neurons are a suitable system for studying both release, and modulation of release by other peptides, of several neuropeptide mediators. (Supported by NSC of Canada.)

873.8


The excitatory amino acids (EAA) glutamate and aspartate and the peptide Substance P (SP) have been proposed as primaryafferent neurotransmitters involved in nociceptive transmission in the mammalian spinal cord. Microdialysis of the extracellular fluid of the dorsal lumbar spinal cord has demonstrated increased concentrations of glutamate and aspartate following formalin injection (Skilling et al., J. Neurochem., 1988), and that the concentration of SP increases after electrical stimulation of the sciatic nerve (Bredt et al., Science, 1987). In the present study, the potential interaction between SP and EAA in the rat dorsal horn was investigated using microdialysis. Male, Sprague-Dawley rats implanted transversely with dialysis tubing through the dorsal spinal cord were perfused with Ringer's solution at 5 µL/min. Samples were collected in 10 min aliquots and analysed for amino acids using HPLC with OPA derivatization and fluorescence detection. Perfusion of the tubing for 100 min produced an immediate two-fold increase in the extracellular concentration of aspartate, but no increase in glutamate, asparagine or glycine. Aspartate concentrations remained elevated throughout the perfusion period. These results demonstrate an interaction between SP and an EAA in a region of the spinal cord associated with primary afferent neurotransmissions. (Supported by USPHS Grants DA049090, DA0190, DA00124, CA13042 and NIDA Training Grant DA07234).
348.11 INTERACTIONS BETWEEN TAURINE AND EXCITATORY AMINO ACIDS: RELEASE COMPARISON OF CORTEX SLICES FROM ADULT AND DEVELOPING MICE. Simo S. Oja and Pirjo Kontro, Department of Biomedical Sciences, University of Tampere, Finland.

Taurine is an important inhibitor of neuronal activity, in the immature brain in particular. Spontaneous and potassium-stimulated (50 mM) release of endogenous amino acids from slices taken from newborn and 1-day-old rats was no different from adult rats. Glutamate release is greatly enhanced by potassium stimulation and is more than 10 times higher in newborn than in adult rats. Taurine is released by potassium stimulation and is more than 10 times higher in newborn than in adult rats. The potassium-evoked release of taurine was potentiated by kainate and NMDA, which effects were antagonized by glycine and D-aspartate, respectively. The receptors for excitatory amino acids thus modify taurine release, particularly in the immature brain.

Supported by the Academy of Finland and the Emil Aaltonen Foundation.

348.12 N-methyl-D-aspartate (NMDA)-induced depolarizations and NMDA - serotonin interactions in old rat neocortex. A. Banksy, J. M. Reynolds and P. J. Carlen, Playfair Neurosci. Unit, Toronto Western Hospital, Addiction Research Foundation, Deps. of Medicine and Physiology, Univ. of Toronto, Toronto, Ont. MST 22B, Canada.

We examined NMDA-induced depolarizations in layer V neocortical neurons in the rat neocortex. NMDA (0.5 mM) was pressurally injected into the recording site. In response to NMDA, the neurons had no correlation between the increase in the NMDA-induced depolarization and the magnitude of the response. Usually, large and long-lasting membrane depolarizations could be evoked in all of the neurons by a marginal increase in NMDA droplet size. When added to the perfusate, serotonin (10 µM) significantly enhanced NMDA-induced depolarizations in young neurons but not in old. It is hypothesized that this lack of serotonin-induced GABA interaction may be related to the loss of synaptic plasticity (long-term potentiation) in old rat neocortex.

Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.


Glycine (GLY), glutamate (GLU), and acetylcholine (ACh) microinjected into the nucleus tractus solitarius (NTS) of rat decrease arterial pressure (AP) and heart rate (RR). GLY has been demonstrated to release ACh from central tissues and to act at the NMDA GLU receptor subtype. Next, we sought to determine if 55 anesthetized rats if the response to microinjection (25 nmol) of GLY was mediated through ACh or GLU mechanisms. Atropine (30 µmol), but not hexamethonium (1 µmol-100 µmol), blocked the response to GLY (100 µmol). Atropine increased the duration of the GLY response by 3.1%; and GLY (100 µmol) significantly increased the response to ACh (250 µmol) from a fall of AP of 11.6 ± 2.3 mmHg before GLY to 17.8 ± 3.9 mmHg after GLY. In contrast, GLY (100 µmol) decreased the AP response to NMDA by 17% and in high doses (100 µmol) blocked the response to NMDA, GLU and ACh. Direct NMDA antagonism did not significantly change the response to GLY; and strychnine, which blocked the response to GLY, did not significantly affect the response to NMDA (3 pmol). These data suggest that GLY may elicit an excitatory-like response in the NTS through release of ACh and not through action on the NMDA receptor.

Supported by HL32205, HL14388, NS24621, Merit Rev. Tab 18.
349.3

Dopaminergic Regulation of Striatal Tachykinin Gene Expression. D.M. Haverstick, D. Beckstead, Dept. of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425.

An influence of the corticostriatal projection on striatonigral substance P (SP) cells was assessed by quantitatively radioimmunocytochemistry (RIC). In rats, cerebral cortex was removed by suction in one sphere from the frontal pole to the level of breata. After 1 or 3 weeks (W=2), the brains were fixed, cut at 30 µm, and mounted onto slides. Sections were incubated in a monoclonal antibody to SP, followed by a biotinylated secondary antibody. The stain was visualized using H3-biotin/mg tissue equivalent by interpolation on a standard curve. SP levels were compared between the contra- and ipsilateral SN following corticostriatal ablation and revealed an increase in ipsilateral nigral SP of 8.83% and 6.19% (p < 0.005) at, respectively, 1 and 3 weeks survival. In a related experiment, striatal glutamate was depleted by more than 80% by 14 days of continuous intraventricular administration of methionine sulfoximine. Radioimmunocytoassay of SP revealed an increase in SN of 18% when compared to vehicle injected controls. The results suggest that corticostriatal (glutamate?) transmission plays a role in the regulation of SP levels in strionigral neurons. Supported by NSF grant BNS 850438.

349.4


Preprotachykinin (PPT) mRNA content of rat basal ganglia is acutely increased following a single i.p. injection of methamphetamine (METH). PPT mRNA contains exons encoding both neuropeptides substance P and substance K. In situ hybridization protection experiments were undertaken to determine if METH treatment induced stimulus-specific alterations in the splicing of PPT mRNA and thus any changes in the ratio of substance P to substance K since tissue-specific PPT splicing has been reported to provide tissue-specific products. The results showed that there are two major species of PPT mRNA, they increase in a parallel manner following METH, and such an increase is blocked by pretreatment with haloperidol. By determining the amount of protectable RNA in the nuclear and cytoplasmic fractions of striatal cells it was possible to show that the METH-induced increase in biosynthesis of substance P is dependent upon nuclear events such as increased gene transcription, hnrRNA processing or mRNA stabilization.

349.5

INFLUENCE OF THE CORTICOSTRIATAL PROJECTION ON SUBSTANCE P CELLS OF THE CORTEX, STRIATUS, AND NIGRA. D. Beckstead. Dept. of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425.

An influence of the corticostriatal projection on striatonigral substance P (SP) cells was assessed by quantitative radioimmunocytochemistry (RIC). In rats, cerebral cortex was removed by suction in one sphere from the frontal pole to the level of breata. After 1 or 3 weeks (W=2), the brains were fixed, cut at 30 µm, and mounted onto slides. Sections were incubated in a monoclonal antibody to SP, followed by a biotinylated secondary antibody. The stain was visualized using H3-biotin/mg tissue equivalent by interpolation on a standard curve. SP levels were compared between the contra- and ipsilateral SN following corticostriatal ablation and revealed an increase in ipsilateral nigral SP of 8.83% and 6.19% (p < 0.005) at, respectively, 1 and 3 weeks survival. In a related experiment, striatal glutamate was depleted by more than 80% by 14 days of continuous intraventricular administration of methionine sulfoximine. Radioimmunocytoassay of SP revealed an increase in SN of 18% when compared to vehicle injected controls. The results suggest that corticostriatal (glutamate?) transmission plays a role in the regulation of SP levels in strionigral neurons. Supported by NSF grant BNS 850438.

349.6

OPIOD PEPTIDES AND SUBSTANCE P. C. Roozendaal, L. Heilbronner, Dept. of Pharmacology, University of South Carolina, Columbia, SC 29208.

Several opioid peptides, SP, their precursors, intermediate-sized precursors, and endogenous precursor-processing enzymes were found in the rat brain using a combination of RIA, radioimmunoassay (RRA) and MS/MS. Methionine enkephalin (ME) and SP were identified by RIA, MS, and MS/MS in pooled human CSF following HPLC separation. Beta-endorphin (BE) immunoreactivity was measured in the appropriate HPLC fractions. Dynorphins and the C-terminal extension of ME were analyzed by using hydrolysing appropriate HPLC fractions followed by ME-RIA and LE-RIA. A fraction at 84 min in a 90-minute HPLC gradient was separated into three fractions with a 120-min HPLC gradient. These fractions were analyzed with human CSF precursor-processing enzymes. ME-RIA and ME-RIA indicated the presence of precursors to ME and BE and of their processing enzymes. Using endopeptidase identification, the HPLC purified precursors were cleaved by CSF enzymes, the products were separated by HPLC: peptides in the HPLC fractions were determined by RIA and then identified by MS/MS. ME precursor and precursor-processing enzymes were proved strongly by MS/MS. The presence of a SP precursor and its processing enzymes was also established by RIA.

349.7

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) PRECURSOR MESSAGE-ENCODED IN BRAIN AND PERIPHERAL TISSUE OF RATS. J.Y. Yang, W.-L. Liu, and A. Y.C. Shum. Dept. of Physiology, National Defense Medical Center, and Dept. of Pharmacology, National Yang-Ming Medical College, Taipei, Taiwan, ROC.

Vasoactive intestinal polypeptide (VIP) is a widely distributed multifunctional neuropeptide. Northern analysis and dot blot hybridization were used to examine the biosynthesis of VIP. A Bluscribe M13 plasmid containing an insert of 1319 bp cDNA encoding rat VIP precursor reported by Dr. T. Middelton, NIMH was transformed into HR6 and which was then transferred in M9 medium. The replicated plasmid was isolated from Er co lysates. A 1400 bp fragment was separated from HR6 containing the Rat restriction digest of the plasmid and nick-translated with [32P]dATP to serve as the probe for hybridization with pre-pro-VIP mRNA. This probe was then electrophoresed onto nitrocellulose by vacuum filtration, then baked in a vacuum oven and hybridized to the same probe. The dot autoradiograms were then scanned densitometrically to quantitate mRNA. The signal in brain and intestine is at least 10-20 fold more intense than in heart and 50 fold more intense than in kidney. Experiments are underway to examine the regulation of VIP biosynthesis during the hypertensive and ischemic states. (Supported by Academia Sinica, ROC)

349.8

STERIOD HORMONE REGULATION OF BRAIN NEUROPEPTIDE Y mRNA LEVELS. P Camp and J.O. W. Davis. Dept. of Pharmacology, Univ. of Tennessee, Memphis, TN 38163.

Estradiol (E2) and progesterone (P4) can induce or block LH surges in ovariectomized (OVX) rats by acting within the brain. Neuropeptide Y (NPY) can also alter central nervous activity and is considered a candidate neuroactive peptide involved in the regulation of LH surges. The effects of prolonged exposure to E2, P4, and their combination on the regulation of NPY mRNA levels during the estrous cycle was studied. In the rat, ovaryectomy was induced on day 0, 1, 2, 3 and 5. Brains were dissected and rapidly cooled at 4 C. Hypothalamic NPY mRNA levels were determined by a competitive inhibition of NPY receptor binding. In our experiments, the presence of a SP precursor and its processing enzymes was also established by RIA.
Neuropeptide Y (NPY) is a peptide hormone that is synthesized in the mammalian brain and is involved in various physiological processes. It is synthesized in the periphery and is found in the central nervous system (CNS), where it is involved in the regulation of appetite, energy metabolism, and memory.

In the CNS, NPY is synthesized by neurons and is primarily localized in the hypothalamus. The synthesis of NPY is regulated by various factors, including cholinergic input, and changes in NPY expression can be detected in response to these stimuli.

NPY is synthesized from its glycine-extended precursor. A bovine NPY cDNA encodes a 108 kD protein with an N-terminal catalytic domain, with a putative transmembrane domain near the C-terminus. Post-translational processing of the precursor and N-terminal amino acids may yield a soluble form of NPY. The levels and forms of NPY mRNA in rat tissues were determined by Northern analysis using bovine NPY cDNA probes. The level of NPY mRNA was highest in the hypothalamus with moderate levels in the cerebral cortex, striatum, hippocampus, thalamus and retina; low levels were found in the olfactory bulb and cerebellum. Heart atrium contained the highest level of NPY mRNA of all tissues; high levels were also identified in adrenal medulla and granulosa cell of the pituitary gland, and in heart ventricle. Moderately high levels of NPY mRNA were found in the submaxillary, sublingual and thyroid glands, while low levels were found in the ovary, adrenal gland, lung and kidney. The ratio of soluble to membrane-associated NPY enzymatic activity was tissue specific.

The synthesis and release of NPY are regulated by various factors, including neurotransmitter function. SRIF biosynthesis can be studied in vivo by injecting labeled cysteine into the cerebral ventricle and measuring the radioactivity in the hypothalamus following 35S-cysteine administration into the third ventricle. This technique has been used to study the regulation of NPY expression in the cerebral cortex following various stimuli, such as cholinergic input or hormonal changes.
A CRITICAL ANALYSIS OF THE USE OF CYSTEAMINE TO DEPLETE HYPOTHALAMIC S-Luteinizing Hormone-Releasing Hormone (SRIF-LI) FOLLOWING SC CNS ADMINISTRATION.

P. F. M. Crother, G. Bissette, and C. B. Cook.

The wide CNS distribution of SRIF-LI and its reduction in Alzheimer’s disease have stimulated studies of its extrahypothalamic role. The extrahypothalamic reduction of hypothalamic SRIF-LI by subcutaneously (SC) administered hypothalamic LHRH (CTH) is well-documented, but reports describing effects of centrally administered CTH on hypothalamic SRIF-LI are discordant. SRIF-LI was measured by RIA in the frontal cortex (CTX), hypothalamus (HYP), and hippocampus (HIP) in rats following daily (x7) infusions into stereotaxically positioned, unilateral cannulae in either the lateral ventricle (LV, 300 µg/µl) or the dorsal HIF (100 µg/µl), and following single (300 µg/kg) or daily (100 µg/kg, x7) SC injections; rats were killed 6 or 24 hr after the last injection. Following LV infusions, SRIF-LI was reduced only in the HYP (35 ± 4 hr and 24 ± 6 hr; p < 0.05). Following HIp infusions, SRIF-LI was reduced only in the HYP at 24 hr (31%) and was not changed in either the ipsilateral or contralateral HYP or CTX. Although the neonatal SRIF-LI by SC CTH was negligible and was not enhanced by repeated dosing, the extrahypothalamic reduction of SRIF-LI by SC CTH was not affected by a particularly effective agent for the reduction of extrahypothalamic SRIF-LI following SC or CNS administration. (Supported by NIH MH-350-245 and NIA AG-05128)

MESSENGER RNA REGULATION III

350.1 ANTI-DEPRESSANT DRUGS INCREASE GLUCOCORTICOID RECEPTOR mRNA IN PRIMARY CULTURES OF RAT BRAIN NEURONS.

H. Akagi and R. Miledi. Laboratory of Cellular and Molecular Neurobiology, Department of Psychology, University of California, Irvine, CA 92717.

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, indicated by elevated serum cortisol levels and a non-suppression of serum cortisol following dexamethasone (DEX) administration in stress response, are frequently associated with major depressive illness. The hyper-cortisolaemia of depression is not associated with any of the physical changes that accompany the illness, and this apparent lack of sensitivity to glucocorticoids may be due to a reduction in glucocorticoid receptor (GR) number. A decreased number of GR in lymphocytes of depressed patients has been noted and, if this decrease is found in the CNS, this could explain the lack of feedback regulation characteristic of non-suppressors. Since non-suppressive responses of serum cortisol to dexamethasone and to normal daily dose anti-depressants therapy, it is possible that anti-depressant drugs modify the GR content of brain areas involved in control of the HPA axis. To test this hypothesis, we investigated the effects of anti-depressants on GR mRNA in cultured rat brain neurones.

A critical analysis of the use of cysteamine to deplete corticotrophin-releasing hormone (CTH) in the hypothalamus of rat brain is presented. Though CTH is barely detectable in the hypophyseal-adenohypophysis, this pituitary gland involved in the control of the HPA axis, and suggest a mechanism for action of their normalisation of endocrinological parameters in depressed patients.

350.2 GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR mRNA EXPRESSION IN RAT BRAIN. H. M. Chao* and B. S. McEwen.

(Spon: A. Miller) Lab. of Neuroendocrinology, Rockefeller Univ., N.Y., N.Y.10021

The action of corticosteroid receptors by glucocorticoids is well documented. The binding of CORT in the rat brain is mediated by two receptor types, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Molecular probes for the GR and MR were used to study the regulation of these receptors at the mRNA level. GR mRNA expression was quantified using a rat GR probe (from R. Evans). Though the MR is barely detectable in intact rats in the cytosolic receptor binding assay, the MR mRNA is readily measured and shows a higher level of expression in the hippocampus than in the other four brain regions examined. Initial indications are that the steady-state level of expression of the MR mRNA (like that of the GR mRNA) is not altered by ADX or CORT treatment. (Supported by MH41256 and NS07080.)

350.3 TWO TYPES OF GLYCINE RECEPTORS EXPRESSED IN XENOPUS OCYCTES INJECTED WITH RAT SPINAL CORD mRNA. H. Akagi and R. Miledi, Laboratory of Cellular and Molecular Neurobiology, Department of Psychology, University of California, Irvine, CA 92717.

Poly(A)-messenger RNAs (mRNAs) isolated from adult and neonatal (3-4 day old) rat spinal cord were injected into Xenopus oocytes which were subsequently examined under voltage clamp. Messenger RNAs obtained from both sources induced the oocytes to acquire functional glycine receptors (GlyR). The glycine-induced currents reversed direction at about -20mV (close to the equilibrium potential of CI in the oocytes) and the GlyR responses were blocked by strychnine (0.5µM) or picrotoxin (20µM). Thus, the GlyR encoded by two kinds of mRNAs share some common characteristics. Nevertheless, size fractionation of the GlyR-mRNAs by sucrose density sedimentation exhibited contrasting profiles; the majority of the adult cord GlyR-mRNA sedimented in heavy density fractions, close to the position of 28 S RNA, whilst the neonatal GlyR-mRNA was seen mainly close to that of the 18 S RNA. The properties of GlyR encoded by the adult mRNA were different from those encoded by the neonatal mRNA in respect to dose-response relationship, time-course of desensitization and some pharmacological actions. These results suggest that in rat spinal cord there exist, at least, two molecular classes of mRNAs which code for distinct glycine receptors. Moreover, the production of the GlyR-mRNAs in the spinal cord appears to be developmentally regulated.

350.4 REGULATION OF TYROSINE HYDROXYLASE (TH), PHENYLTHIOETHANOLAMINE N-METHYLTRANSFERASE (PNMT) AND PROENKEPHALIN (pEK) mRNAs IN ADRENAL MEDULLARY CELLS BY CATECHOLAMINE DEPLETING AGENTS. ROLE OF TRANSCRIPTION AND PROTEIN SYNTHESIS.


Depletion of catecholamines (CA) in cultured bovine adrenal medullary cells (BAMC) increases the content of TH and pEK derived peptides and modifies TH, pEK and PNMT mRNA levels. Incubation of BAMC with tetranebazene (TBZ), 0.1-100 µM, for 48 hr produced a dose-dependent depletion of CA and a inversely proportional increase in TH and pEK mRNA levels. Reserpine produced similar results suggesting that the levels and/or intracellular distribution of the CA regulate the expression of TH and pEK genes. The level of PNMT mRNA exhibited complex responses to TBZ and reserpine. Increases (+50 to +100%) were observed at low concentrations of the drugs but decreases (-40 to -90%) at the higher concentrations. TBZ at 100µM produced biphasic time-dependent changes in pEK mRNA; a 2-3 fold increase at 3 hours followed by the 1h an -90% at 48 hours. The increase in TH mRNA (+40%) by TBZ was not affected by cycloheximide (+40%) but reduced to -82% in the presence of a-samitin (both drugs at 10µg/mL), suggesting transcriptional regulation of TH. In contrast, both the increase in pEK and the biphasic responses of PNMT mRNA after CA-depleting drugs, were prevented by cycloheximide, indicating involvement of protein synthesis in the regulation of these two adrenomedullary proteins.
350.3

To study the effect of prolonged neuronal activity, we examined the effect of long-term treatment of PC12 cells with cocaine. Cells were treated for 3 days with 55 mM KCl. Morphologically, the general cell appearance in phase contrast microscopy was unaltered. The cellular dopamine and norepinephrine were completely depleted, as expected. Total RNA was isolated from the cells and analyzed by Northern blot analysis, and hybridized with a 32P-labeled cDNA to tyrosine hydroxylase (TH). The TH mRNA level was decreased by 10-15%.

The time course of this effect showed that the TH mRNA levels were not decreased upon treatment for 1 day or less with 55 mM KCl, and by day 3, the decrease in TH mRNA was significant (greater than 10-fold).

The specificity of this effect was examined by assessing actin mRNA levels and total protein synthesis under these conditions. Hyrbridization with actin cDNA showed no decrease in actin mRNA following this treatment. Moreover, protein synthesis, measured by incorporation of [35S]-methionine into protein, was similar at these time points. Thus, there appears to be a long specific decrease in TH mRNA upon prolonged treatment of PC12 cells with 55 mM KCl. (Supported by NIH grant NS 20440)

350.4

Dopamine- and adenosine-A3:5'-nucleotide-sensitive-phosphorylated protein (DARPP-32) is highly enriched in neurones of the caudate nucleus. We investigated the effects of dopamine-receptor agonists on steady-state levels of DARPP-32 mRNA. In control animals, the distribution of DARPP-32 mRNA correlated with that of the protein as previously shown by immunocytochemistry. After overnight autoradiography, a strong signal was observed in the caudate nucleus, particularly in the mediodorsal neurones of the caudate nucleus. The experiments were performed on the caudate nucleus in order to be able to distinguish DARPP-32 mRNA from other mRNA species expressed in the caudate nucleus. The present study was designed to investigate whether or not DARPP-32 mRNA is expressed in the caudate nucleus.

We have recently reported that both cAMP and glucocorticoids induce levels of tyrosine hydroxylase (TH) in PC12 cells, and that induction of TH mRNA is increased by cAMP and glucocorticoids. In an effort to explain this effect, we have used a primer extension assay to examine whether, or not, this protein factor(s) is required for increased transcription of the TH gene or acts to stabilize the TH mRNA in PC12 cells.

350.5

Anatomy/Neurobiology and Phynology Dept., Washington University School of Medicine, St. Louis, MO 63110

It has been shown that tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine production, is expressed in various secretory pancreatic islet cells (Tatematsu and Lee, Dev. Biol. 121: 454, 1987). We have recently shown that the human TH gene is within 2 kb of the insulin (INS) gene on chromosome 11. Such close linkage raises the possibility that factors which influence one gene might affect the other.

As a first step in testing this hypothesis, we have isolated rat pancreatic islet cells, maintained them in discrete cultures, and tested for TH or INS mRNA expression using in situ hybridization in conjunction with immunological techniques. Preliminary experiments suggest that both INS and TH gene expression is dependent on glucose concentration. Six hours after placing in media containing 1.65 mM glucose, INS-containing, islet cells expressed high levels of INS mRNA. In parallel cultures, TH mRNA was detected in a subset (80%) of INS immunoreactive cells. The levels of TH mRNA in these cells ranged from 5-90% of the average amount of INS mRNA expressed under comparable conditions. Under low glucose (5.5 m M) conditions, both INS and TH message could be detected at lower levels in islet cells cultured for 14 hours, but after three days, less than 1% expressed INS mRNA, while TH message could not be detected. In contrast, islet cells maintained in 16.5 mM glucose produced both messages after all time intervals. These data suggest that TH and INS are coordinately regulated by glucose. We are currently determining if other factors known to modulate TH gene expression also co-regulate INS levels.

350.6

In the present study we sought to determine if the decrease in TH synthesis was due to decreased transcription of the TH gene in PC12 cells. TH mRNA was determined by in situ hybridization using a [32P]labeled oligonucleotide probe. Densitometric quantification of the resulting autoradiograms showed a 95% decrease in TH mRNA in DA treated cells. In a similar experiment, 2mM epinephrine resulted in a 30% decrease in TH mRNA. Northern blot analysis of mRNA isolated from DA to DA also revealed a 70% decrease in TH mRNA. Thus DA decreases TH synthesis, reducing TH mRNA in cultured adrenal medulla.

350.7

We have recently reported that dopamine (DA) reduces the activity and amount of tyrosine hydroxylase (TH) mRNA in cultured rat adrenal medulla (Biochem. Pharmac. 37, 1391-1398, 1988).

In the present study, we determined if the decrease in TH synthesis was due to decreased transcription of the TH gene in PC12 cells. TH mRNA was determined by in situ hybridization using a [32P]labeled oligonucleotide probe. Densitometric quantification of the resulting autoradiograms showed a 95% decrease in TH mRNA in DA treated cells. In a similar experiment, 2mM epinephrine resulted in a 30% decrease in TH mRNA. Northern blot analysis of mRNA isolated from DA to DA also revealed a 70% decrease in TH mRNA. Thus DA decreases TH synthesis by reducing TH mRNA in cultured adrenal medulla.

We have examined the regulation of POMC gene expression in AM cells using a single-stranded DNA probe complementary to 30 nucleotides of the coding region of POMC. Cells expressing POMC mRNA were unevenly distributed in AM, and were not found in the adrenal cortex. Without brain injury, an increased percentage of the POMC mRNA negative cells were observed in the adrenal cortex. These changes were distributed throughout the mediulla. No changes in the POMC mRNA were detected. Together, these results indicate that the neural regulation of POMC gene expression in AM cells is complex. Under basal condition neural input appears to have an inhibitory effect, whereas elevation of the nerve activity by hypoglycemia enhances POMC gene expression.


Aneurin and intermediate lobe propiomelanocortin (POMC) cells are derived from a common embryological precursor that differentiates differently during development and are characterized by developmental changes in the extent of prohormone processing. In this study we have determined the levels of POMC primary transcript and POMC mRNA in separated anterior and neurointermediate (NIL) lobes at late prenatal and early postnatal stages of rat development using POMC antiserum splice junction probes. Aneurin and NIL lobes were dissected from fetal (el14), neonatal (pl16,pl20), and adult Sprague-Dawley rat and nuclear and cytoplasmic fractions isolated. Radio labelled anti-tissue RNAs were prepared from an SP65 vector containing a rat genomic DNA fragment encoding all of POMC exon 1 and 60 bp of in-frame 30 of all of POMC. Using a labeled sense RNA. Although the amount of POMC primary transcript per pituitary increased in both cell populations during these ages, (e.g. 0.03 fold/anterior pituitary (AP) at e18 to 0.10 fold/AP in the adult), POMC mRNA levels increased by greater amounts in both lobes. Thus, the relative abundance of POMC primary transcript compared to mature POMC mRNA in both anterior and NIL was markedly higher during late gestation than in the adult, which indicates that dynamic changes in POMC processing are occurring during differentiation of both POMC cell populations during development. Supported by NIH grants to SF and J.J.R.


Adrenallectomy has been used as a paradigm for studying the role of glucocorticoid negative feedback on the hypothalamic-pituitary system. However, using this procedure it is difficult to study acute effects, since confounding factors such as surgical stress and steroid release during removal of adrenals are often associated with this procedure. To observe the acute consequences of endogenous glucocorticoid removal, we have employed metyrapone as a potent inhibitor of adrenal steroid synthesis and studied the changes in mRNA levels in relevant nuclei. Rats were treated with daily injections of metyrapone (20mg/100g bw) for 3 days, sacrificed, and the hypothalamus was microdissected using a brain block. Using RNAse protection assay, we observed 200% increase of POMC mRNA over controls in the anterior pituitary, confirming our previous data obtained from dot blots. B. S. McEwen (SPON: G. Ryan). Lab of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

We have investigated the regulation of oxytocin gene expression in the suprachiasmatic nuclei during days 10, 18, and 22 of pregnancy and day 5 of lactation in the rat, using radioactive RNA hybridization. The level of oxytocin mRNA in the PVN, compared to the control, was increased by 200% in late pregnancy and by 300% in late lactation. This increase was not due to an increase in the number of oxytocin containing cells, but rather to an increase in the amount of oxytocin mRNA per cell. In the hypothalamus, the level of oxytocin mRNA was increased by 150% in late pregnancy, but not significantly in late lactation. The increase in oxytocin mRNA was not due to an increase in the amount of oxytocin mRNA per cell, but rather to an increase in the number of oxytocin containing cells. These results indicate that oxytocin mRNA levels in suprachiasmatic nuclei remain relatively constant over late pregnancy, while lactation is associated with an increased percentage of cells in the suprachiasmatic nuclei expressing higher mRNA levels. We are presently determining whether changes in oxytocin cells in other brain regions undergo similar changes.


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We have examined the regulation of PEN gene expression in AM cells using a single-stranded DNA probe complementary to 30 nucleotides of the coding region of PEN. Cells expressing PEN mRNA were unevenly distributed in AM, and were not found in the adrenal cortex. Without brain injury, an increased percentage of the PEN mRNA negative cells were observed in the adrenal cortex. These changes were distributed throughout the mediulla. No changes in the PEN mRNA were detected. Together, these results indicate that the neural regulation of PEN gene expression in AM cells is complex. Under basal condition neural input appears to have an inhibitory effect, whereas elevation of the nerve activity by hypoglycemia enhances PEN gene expression.


In situ hybridization histochemistry was used to evaluate the effects of two pharmacological agents with D2 dopamine receptor subtype antagonistic activity, haloperidol and dopamine, on levels of proenkephalin (PE) mRNA in the caudate-putamen (C-PU) region of the rat brain. Male Sprague-Dawley rats were treated for six days with haloperidol (1 mg/kg/day) or dopamine (0.4 and 0.8 mg/kg/day) delivered by osmotic minipump. A synthetic oligonucleotide (30mer) complementary to regions in the first and second exons of the PE message was end-labeled with [32P] and used as a hybridization probe to detect specific mRNA in coronal brain sections from treated and control rats. Optical density of X-ray film exposed by labeled sections was quantitated in the C-PU region by densitometry using an image analyzer and data were expressed as percent of control levels. Treatment with haloperidol resulted in a 2.5 fold increase in the level of PE mRNA, while treatment with dopamine resulted in decreased levels. The effect of haloperidol on PE mRNA in the C-PU region of the rat brain is not equivalent to the antagonistic effect of dopamine on the D2 dopamine receptor subtype.
350.17
**EFFECT OF SODIUM DEPLETION AND ADRENALCORTICOSTEROID TREATMENT ON SUBSTANCE P NEURONS IN THE RAT**


We have studied the effect of sodium depletion and aldosterone treatment on brain angiotensinogen mRNA by *in situ* hybridization histochemistry using a synthetic oligonucleotide probe corresponding to amino acids 106-115 of pro-angiotensinogen. The probe was radio-labelled at the 3' end by the addition of adenosine mononucleotides (32P) by terminal deoxynucleotidyl transferase.

Sodium depletion resulted in increased levels of angiotensinogen mRNA in the preoptic area and no changes in the dorsomedial and ventromedial nuclei of the hypothalamus or the medial amygdala. Aldosterone treatment increased angiotensinogen mRNA levels in the preoptic area but not in other brain areas examined. The data suggest that sites in the preoptic area of the brain are part of a neural circuit involved in regulating the hunger for salt.

350.19
**EFFECTS OF STRIATAL DOPAMINE DEPLETION ON SOMATOSTATIN NEURONS.**

**T. T. Lu**, J. M. Goldstein, R. H. Roth, F. Baldino, Jr., and A. Cephalon, Inc., West Chester, PA.

Among the intrinsic striatal neurons is a population of cells containing both somatostatin (SOM) and neuropeptide Y (NPY). Previous studies have revealed that depletion of striatal dopamine (DA) results in changes in striatal tachykinin and enkephalin neurons, as reflected by changes in peptide mRNA levels. We have therefore examined the effects of striatal DA depletion on SOM neurons, using immunohistochemical (IHC) and in situ hybridization histochemical (ISHH) methods. Polyclonal antisera to SOM were localized in somatostatinergic neurons of the substantia nigra and the striatum, and the distribution of SOM-like immunoreactivity in the neostriatum was evaluated by in situ hybridization histochemistry. SOM-like immunoreactive neurons were found to be decreased in number by 15-20% in the striatum of DA-depleted animals. The changes in the distribution of SOM-like immunoreactive neurons were not observed in the substantia nigra, where a decrease in SOM mRNA levels was noted.

350.21
**PREPOTACHYKININ A GENE EXPRESSION IN RAT ENTERIC AND SENSORY NEURONS.**


The mammalian tachykinin (TK) peptide, substance P (SP) and neurokinin A (NKA), and neurokinin B (NKB) are encoded by distinct mRNAs derived from separate SP/NKA and NKB genes. Using antisera directed against the conserved TK C-terminal region as general markers for the TK family and immunohistochemistry, numerous immunoreactive cells were identified in both the ganglion cell layer (GCL) and proximal inner nuclear layer (INL) of the retina. In situ hybridization histochemistry with 35S-labelled rat SP/NKA- or NKB-encoding antisense RNAs showed SP/NKA-encoding transcripts in cell bodies distributed to the GCL and proximal INL, and NKB-encoding transcripts in somata located in the GCL. Northern blot analysis confirmed the presence of these mRNAs in retinal extracts. Nuclease protection experiments showed a single NKB-encoding transcript, but multiple SP/NKA-encoding transcripts (gamma/SP/NKA mRNA > beta-SP/NKA mRNA > alpha SP mRNA). These results demonstrate that both TK genes are expressed in the rat retina. The differential localization of SP/NKA- and NKB-encoding mRNAs in the INL and GCL documents a cell-specific expression of TK-encoding mRNAs in the retina. Supported by EY04067, NS21937 and SKB Fellowship.

350.22
**CELLULAR LOCALIZATION OF SUBSTANCE P/NEUROKININ A AND NEUROKININ B mRNAs IN THE RAT RETINA.**


The mammalian tachykinin (TK) peptide, substance P (SP) and neurokinin A (NKA), and neurokinin B (NKB) are encoded by distinct mRNAs derived from separate SP/NKA and NKB genes. Using antisera directed against the conserved TK C-terminal region as general markers for the TK family and immunohistochemistry, numerous immunoreactive cells were identified in both the ganglion cell layer (GCL) and proximal inner nuclear layer (INL) of the retina. In situ hybridization histochemistry with 35S-labelled rat SP/NKA- or NKB-encoding antisense RNAs showed SP/NKA-encoding transcripts in cell bodies distributed to the GCL and proximal INL, and NKB-encoding transcripts in somata located in the GCL. Northern blot analysis confirmed the presence of these mRNAs in retinal extracts. Nuclease protection experiments showed a single NKB-encoding transcript, but multiple SP/NKA-encoding transcripts (gamma/SP/NKA mRNA > beta-SP/NKA mRNA > alpha SP mRNA). These results demonstrate that both TK genes are expressed in the rat retina. The differential localization of SP/NKA- and NKB-encoding mRNAs in the INL and GCL documents a cell-specific expression of TK-encoding mRNAs in the rat retina. Supported by EY04067, NS21937 and SKB Fellowship.

350.18
**BRAIN ANGIOTENSINOGEN mRNA IN GENETICALLY HYPERTENSIVE AND NONHYPERTENSIVE RATS.**

**J. Angulo, G. Angelone, B. B. McEwen.** New York State Psychiatric Institute and Rockefeller University, New York, NY 10021.

Brain angiotensinogen is implicated in blood pressure (BP) control and fluid/electrolyte metabolism, via vasopressor action and stimulation of thirst and salt consumption. Spontaneously Hypertensive rats (SHR) have a higher BP, but also consume more salt than Wistar/Kyoto rats (WKY), a normotensive strain from the same progenitor. SHR also seem to be more sensitive than WKY to the BP and salt appetite stimulating actions of the renin-angiotensin system. We are examining genotype and adrenal influences on expression of the three tachykinins, substance P, neurokinin A (NKA), and substance P-like immunoreactivity, in the brain of SHR and WKY. Brains of SHR and WKY, either intact or adrenalectomized, were frozen and sectioned at 16 μm. Brain angiotensinogen mRNA was detected by *in situ* hybridization histochemistry with a synthetic oligonucleotide probe (proangiotensinogen amino acids 106-115). The probe was radio-labelled by addition of adenosine mononucleotides (32P) at the 3' end by terminal deoxynucleotidyl transferase. Hybridization autoradiograms were quantitated by computerized densitometry. Preliminary results demonstrate a greater expression of angiotensinogen mRNA in circumscribed regions of SHR brain, and that this difference is enhanced after adrenalectomy.
351.3 EPILEPTIC SEIZURES IN A GENETIC MOUSE MODEL OF EPILEPSY: HIPPOCAMPUS AND NEOCORTEX II

Epileptic seizures in rats have been demonstrated to be correlated with hyperexcitability in mossy fibers into the inner molecular layer (IML) of fascia dentata (FD) distribution and density in surgically-excised human hippocampi electrographic/clinical seizure onsets. Cell counts of Nissl-stained (n=13) known to be epileptogenic by prior in vivo recordings of focal 3

NIH Grant NS02808.


Kindling has features that replicate certain forms of human epilepsy. The epileptiform activity induced by kindling is characterized by synchronous hyperexcitability of neurons in this respect it is similar to LTP, a model of learning/memory. Chang and Greenough (Brain Res. 309:35, 1984), have demonstrated an increase of shaft and semele synapses, suggested intermediaries during kindling, following LTP. We adopted an in vitro kindling model from Staszewski et al. (1985). Control or kindling stimulation was administered to the CA1 region of hippocampal slices. The number of synapses in this region was increased by only 0.9% which was not significant. The findings is comparable to those obtained with LTP. These results support the view that kindling and LTP share a similar mechanism of modifying hippocampal circuitry. Our quantitative EM study strongly suggests that synaptogenesis occurs during kindling. These new synapses may participate in feedforward connectivity increasing synchronization. Supported by Epilepsy Foundation of America and ONR

351.5 KAINATE INJECTION INCREASES GAD mRNA LEVELS IN HIPPOCAMPUS (H) and outer layers of neo- and paleocortex within 5 hours after onset of seizure activity. At 24 hours, AH was extensive in these regions, and was also present in amygdala, dorsomedial (DM) and laterodorsal (LD) thalamic nuclei and nucleus reuniens (R); the deep layers of neo- and paleocortex were conspicuously devoid of heightened immunoreactivity. By 1 week following 3-5 hours of status epilepticus, H, DM, LD, R and deep layers of pyriform cortex contained nercrotic cells. The relative amount and distribution of total number of synapses was similar at 1 month but was decreased and more restricted at 4 months after the seizures. These findings indicate that rapid, progressive and long lasting degenerative changes result from a single incident of seizure activity. Supported by NSERC.
ENHANCED ASPARTIC ACID RELEASE FROM HIPPOCAMPAL SLICES OF EPILEPTIC (El) MICE. 
H.J. Flavon, A. Wieraszko, and P.N. Boyett. Dept of Biology, Boston College, Chestnut Hill, MA 02167.

The Epileptic (El) mouse represents a natural, genetic model of epilepsy. An increase in synaptic plasticity, both synaptic and axonal, but seizures can also be induced by twirling the animal by the tail. The release of putative neurotransmitters (Asp, Glu, and GABA) was studied in hippocampal slices from El mice, and five age matched non-epileptic C57BL/6J (B6) mice. The average number of observed seizures per El mouse was 86. Slices of hippocampal slices (400 µm thickness) were incubated in a 400 ml buffer, either in the presence or absence of calcium. The amount of neurotransmitter released per sample was expressed as a percent of the total release from a given set of slices.

For each transmitter, the basal release was subtracted from the potassium stimulated release in the presence and the absence of calcium. These differences were used to characterize the neurotransmitter release in each mouse strain. The data were expressed as the mean ± SEM. No differences were found between the El and B6 mice (18 ± 0.6% vs 24 ± 2.6% of the release of Glu (P > 0.05) or GABA (25.0 ± 4.6% vs 24.1 ± 4.1%)).

Asp release, however, was significantly higher in the El mice (39 ± 7.2 %) than in the B6 mice (31.4 ± 2.8 %, P < 0.01). Thus, enhanced Asp release may be related to seizures. Supported by the Michael P. Walsh Scholarship, and grants from NSF (NSF 804955) and NIH (23355, 24286).

351.10 RELEASE OF EXCITATORY AND INHIBITORY AMINO ACIDS FROM HIPPOCAMPAL SLICES IN TETANY TOXIC EPILEPTIC MICE.

Mice were given a single dose of pentylenetetrazol (PTZ) in order to induce seizures. The release of excitatory and inhibitory amino acids (Glu and GABA, respectively) was measured from hippocampal slices of mice that were either normal or had been given PTZ (24 mg/kg IP) repeated every other day initially and every day subsequently. The release of amino acids was measured in the presence and absence of calcium. The results show that the release of Glu is significantly higher in the PTZ-treated mice (33.0 ± 2.6 / 34.8 ± 4.1) than in the control mice (30.7 ± 2.4 / 31.0 ± 2.6). Asp release, however, was not significantly different between the groups.

Supported by the Welcome Trust and N.E.C.

351.11 PRESENCE OF INTRINSIC EPILEPTIFORM ACTIVITY IN HUMAN BRAIN SLICES.

Electrophysiological properties of neurons in the superficial layers of human temporal neocortex were studied using intracellular recordings in 34 neonatal, human, and experimental animals. These studies were performed in 34 neonatal, human, and experimental animals. Neurons were recorded from the superficial layers of human temporal neocortex using intracellular recording techniques. The majority (32/34) behaved like regular spiking neurons. They displayed resting membrane potential of 75 ± 1.4 mV (mean ± S.E.; n=32) and 35/34 exhibited the K⁺-evoked, Ca⁺⁺-dependent release of amino acid transmitters into the superfused hippocampal slices, as well as a Ca⁺⁺-dependent spontaneous activity. We measured the release of endogenous aspartate, glutamate and GABA by HPLC, and of preloaded radiolabeled D-aspartate and GABA by liquid scintillation counting. The effects of K⁺, Ca⁺⁺, experiment number, and injection type were assessed using analysis of variance. Both glutamate and GABA showed a K⁺-dependent, Ca⁺⁺-evoked release, but radiolabeled D-aspartate and endogenous aspartate did not. The release of GABA was impaired by the tetanus toxin treatment.

We conclude that: (1) radiolabeled D-aspartate is not a suitable marker for glutamate as a transmitter; (2) endogenous aspartate does not behave like a transmitter in this experiment; (3) transmitter release is impaired by the minute dose of intra-hippocampal tetanus toxin needed to induce a characteristic hippocampal seizure.

Supported by the Welcome Trust and N.E.C.
MOSSY FIBER LESION DOES NOT PROTECT CA3 HIPPOCAMPAL PYRAMIDAL CELLS FROM EXPERTLY RELEASED EPSPs. (Supported by NIH grant NS 12151 and the Pimley Fund.)

SYNAPTIC INHIBITION AND THE PRODUCTION OF Ictal DISCHARGES BY CHOLINERGIC AND ANTICHOLINERGIC AGENTS IN RAT HIPPOCAMPUS. F.L. Lebeda, T.H. Ton*, and J.B. Ratsik. Sct. of Neurophysiol. Prag. in Neurosci., Dept. of Neurol., Baylor College of Medicine, Houston, TX 77030.

A variety of convulsants (e.g., picrotoxin (PTX), 4-aminoimidazole) produce brief (20-200 ms), synchronous, repetitive discharges in hippocampal slices whether or not the agent interacts with GABA. Cholinergic agonists (acetylcholine, carbachol), study was conducted to determine the role of GABAergic transmission during the epileptiform activity induced by partial (bethanechol, oxotremorine and pilocarpine) and full (acetylcholine) muscarinic agonists (1-150 µM) as well as irreversible anticholinesterases (anti-ChE; DFQ or soman; 10-25 µM).

Besides producing brief (interictal) events within 30 min of exposure in the CA3 subfield, prolonged exposure (1-2 h) to these agents could produce repetitive ictal discharges — extracellularly recorded events lasting >2 s. A transition from interictal to ictal discharges also occurred within 30 min with the co-application of salines containing 7.5 mM K+ or 5-10 mM Li+ . The ictal discharges were characterized by a prolonged voltage-dependent depolarizing component and often by an absence of a visible fast IPSP.

To block all spiking activity and the underlying intrinsically generated discharges, which are not blocked at membrane potentials positive to rest. More than one burst or repetitive discharges were generated during a depolarizing current pulse. Synaptic responses are characterized by a prolonged voltage-dependent depolarizing component and often an absence of a visible fast IPSP.

Supported by NIH grant NS 131809; NR02984


Polysonaptic excitatory interactions are under investigation. (Supported by AFOSR DAMD17-86-C-6029, DAMD17-86-C-360, DAMD17-86-C-6029, AFOSR 85-0118, NIH grants NS 11535 and 10649, and the Klingenstein Fund.)
NEUROTOXICITY IN DEVELOPMENT II

352.2 SYPTOMATIC EFFECTS OF CHRONIC PERINATAL ETHANOL EXPOSURE DEMONSTRATED IN MICE. J. L. Anciano, N. A. Daniel, and G. R. McLean. Department of Pharmacology, University of California, San Francisco, CA 94120. We have previously investigated the effects of ethanol on rat brain development in utero and postnatally. In the present study, we examined the effects of chronic ethanol exposure on mouse brains. Mice were exposed to ethanol via their dams during gestation. The brains of the pups were analyzed for several parameters, including the number of neurons and glial cells. The results showed that chronic ethanol exposure during gestation resulted in a significant decrease in the number of neurons and glial cells in the developing mouse brain. These findings indicate that chronic ethanol exposure during gestation has a significant impact on brain development and may contribute to neurodevelopmental disorders in humans.

352.3 IBOTENIC ACID INDUCED DEMYELINATION AND INFLAMMATORY RESPONSE. R. J. Coffey, W. H. Perry, and J. N. Katz. Department of Experimental Psychology, University of Oxford, England. Demyelination induced by ibotenic acid within the central nervous system coincides very closely with the borders of areogen containing a high density of leucocytes (Coffey et al., Neurosci Lett., 64: 178, 1986). To assess further the correlation between the extent of the recruited haemopoetic cells and demyelinisation after ibotenic acid injections, rats were exposed to either 700 rad, 900 rad, or 1000 rad with a lead shield covering the head or no irradiation, and were then given ibotenic acid injections (0.21 µl of 10 µg/µl) into the medullary septum. The number of neuronal cells and non-neuronal cells was counted in the region of the lesion. Demyelination was measured by an independent observer in adjacent sections. This study showed that there was concomitant reduction in demyelination and cell recruitment after exposure to ibotenic acid, but no change in neuronal loss. These results support the view that demyelination following ibotenic acid injections is due to a non-specific or 'bystander' effect of the inflammatory response. The integrity of the blood-brain barrier will also be discussed.

352.4 IMMUNE SYSTEM RESPONSE TO STERILE LESIONS IN RAT BRAIN. H. Akisan, S. Itagaki, P. L. Weiler, and W. O. McLean. Department of Pharmacology, University of British Columbia, Vancouver, Canada, V6T 2W5. Expression of major histocompatibility complex (MHC) class I and class II surface glycoproteins was observed immunohistochemically in rat brain following sterile lesions. These were induced by epidural kainic acid application, lateral hypothalamic 5-hydroxydopamine injections, or intracortical needle cuts. Both MHC class I and class II antigens were expressed by microglia outnumbered class II antigens, but many expressed both MHC class I and II antigens. These data indicate a selective synaptic pathology by CPEE. (Supported by DA-0599, NIDA)
352.5 NEONATAL EXPOSURE TO THE LIMICRIS-SYSTEM NEUROTOXIN, TRICHLOROPHENOL, INDUCES HEMORRHAGE IN PREGNANTING RATS. C.V. Pickens* and M.E. Stanton. (SPON: M.I. Cage). Northrop Services, Inc. and Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711.

Neonatal exposure to TMD causes a loss of hippocampal pyramidal cells and impairs cognition in adult animals. Relative rat survival after the known effects of neonatal TMD on cognition in infant animals. Here we report that TMD treatment potently alters delayed-alternation learning in 18-day-old rat pups.

Long-Evans rat pups received an i.p. injection of either TMD (5 ml/kg) or vehicle on postnatal day ten (PND 10). These pups were then tested on PND 18 for their ability to learn two tasks in a T-maze. One task was discrete-trial delayed-alternation (working memory) and the other was a simple spatial discrimination (reference memory). Testing occurred on PND 18 because this appears to be the age at which normal rat pups can first perform the alternation task (see Green and Stanton, Behavioral Neurocognition, in press, for results and methodological details). TMD transiently impaired learning, but not asymptotic performance, of spatial discrimination, but utterly abolished learning of delayed alternation.

These results indicate that TMD can be shown to impair cognition during development; and suggest a role for limbic system maturation in the ontogeny of working memory.

352.7 NEOVASCULARIZATION IN THE KAINIC ACID-INDUCED LESIONS OF RAT STRIATUM. AN IMMUNOHISTOCHEMICAL STUDY WITH LAMININ. K. Shigematsu*, H. Kamada*, I. Akiyama* and M. Kameya*. (SPON: D. Martin). Dept. of Neurology, Faculty of Medicine, Kyoto University, Kyoto 604, Japan.

Vascular changes that follow stereotaxic injection into the rat striatum of kainic acid, excitatory neurotoxin, were studied at different time intervals after the lesion by laminin immunohistochemistry. 2 µg of kainic acid was dissolved in 1 µl isosmotic saline and was injected into the striatum of male rats weighting approximately 300g of the Wistar strain. At various times after treatment (2, 7, 28 days and 3 months) the animals were sacrificed by transcardiac perfusion with a fixative containing 4% paraformaldehyde under deep anesthesia. The brains were removed and sections 20 µm thick were prepared on cryostat. Tissues were stained for laminin, tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) using standard immunohistochemical procedures. Immunohistochemistry using antibodies against laminin, a basement membrane glycoprotein, revealed striking neovascularization in the lesioned striatum. The increased laminin-immunoreactivity remained at least up to 3 months after the operation. Rapid and profound changes in striatal astrocytes were demonstrated by increased GFAP-immunoreactivity, whereas TH-immunoreactive axons in the injected region did not markedly differ from the control.


Enriched rearing attenuates the performance deficit induced by neonatal MSG injections in the rat. Male Wistar rat pups were cross fostered at birth and injected daily with MSG (20 mg/kg) from postnatal day (PND) 0 to 6. Control pups received an equivalent volume of saline. Following weaning at 25 days the rats were randomly allocated to enriched or isolated rearing to a 5/4 treatment (groups of 10 rats/sex; Enriched-Saline-Enriched-Saline-Isolated). After 35 days in their respective rearing environments all rats were weighed and tested in an open field activity test (OFT) and a one-trial active avoidance task (AAV). Rats raised in the enriched environment had a significant reduction in total distance traveled compared to their isolated counterparts. Rats raised in the enriched environment also showed a significant improvement in the ability to learn the active avoidance task.


Polychlorinated biphenyls (PCBs) are often contaminated with the more toxic polychlorinated dibenzofurans (PCDFs). Furthermore, municipal and/or 20 ppb PCDFs (equivalent to the PCDF contamination found in the original PCB mixture) for 28 d. Following exposure, the animals were sacrificed, their brains rapidly removed, dissected and homogenized in ice-cold perchloric acid + EDTA. Supernatants were analyzed by HPLC. Coronal 2-mm sections corresponding to plates A23 to A25 were cut. The two-thirds region (medial to lateral) from the caudate (CA), putamen (P) and globus pallidus (GP) were homogenized in ice-cold perchloric acid + EDTA. Supernatants were analyzed by HPLC.

NHS rats received an i.p. injection of kainic acid, an excitotoxic agent, daily to corn-oil (controls) or corn-oil + PCBs (Agrawal et al., 1981; Seegal et al., 1986). However, doses used were greater than those that induce behavioral changes in monkeys (Rosen et al., 1978; 1981).

To determine whether low-dose PCBs alter CA function in a species neurochemically similar to humans, adult male Long-Evans rats (pig-tailed macaque) daily to corn-oil (controls) or corn-oil + PCBs (Agrawal 1980, 400 to 600 µg/kg/day) for 36 weeks. Rats were then deeply anesthetized and sacrificed by decapitation. Brains were removed, chilled in saline, frozen in dry-ice and stored at -80°C. Coronal 2-mm sections corresponding to plates A23 to A25 of Winter et al. (1969) were cut. The two-thirds region (medial to lateral) from the caudate (CA), putamen (P) and globus pallidus (GP) were homogenized in ice-cold perchloric acid + EDTA. Supernatants were analyzed by HPLC.

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neurons were found in synaptic contact with unlabeled spines, denoting the ipsilateral substantia nigra (SN). Grafts contained 100-700 normal mouse embryos were implanted to the striatum of adult rats. At 30 days after grafting, host striata were deprived of the intrinsic tyrosine hydroxylase (TH) immunoreactive neurons. TH axon terminals were seen in the physiological state of host striatal neurons. Following a 3-10 week survival period, animals were perfused with 4% paraformaldehyde. Sections (40 µm thick) were incubated in rabbit antibodies to either leucine enkephalin (1/10,000), serotonin (1/3000), substance P (1/10,000) or neurotensin (1/7500) (ImmunoNuclear, Stillwater, MN) containing 1.0% normal goat serum and 1.0% Triton X-100 in PBS. The immunoreactivity was visualized using either the ABC technique (Vector Labs.) or PAP technique (Cappel) with DAB as the chromogen. Localization of leucine enkephalin, substance P, neurotensin- and somatostatin-like immunoreactivity in the septal region following a KA lesion indicated that all four transmitters were present in axons and terminals within the lesion sites and the staining appeared more intense in the unoperated controls than in the lesioned groups. These results were supported by NIH grant NS22766.

Supported by NIH Grant NS22766

352.13 THE EFFECTS OF ASPARTAME INGESTION ON THE DEVELOPMENT OF THE VISUAL SYSTEM IN THE GUINEA PIG D.A. Buehler, L.A. Sobey, D. & D. Dow-Evans, Laboratory of Cerebral Metabolism, Dept. of Neurosurgery, State University of N.Y., 11203.

Aspartame (Nutrasweet) is commonly used by pregnant women as an artificial sweetener. Once ingested, aspartame is metabolized into phenylalanine, aspartic acid and methanol. Methanol is known to be toxic to the visual system in the adult guinea pig. Therefore, we have investigated the toxicity of aspartame with regard to the developing visual system in the guinea pig. Virgin Duncan-Hartley guinea pigs were time mated in our laboratory. We administered aspartame at 500, 250, or 0 mg/kg body weight in sesame oil by mouth between day 1 of gestation and day of birth. A non-treated control group was also maintained. At the 55th day of gestation some animals were sacrificed, fetal orbits were removed, fixed in buffered formalin, and sectioned for histopathological analysis. Thickness of retinal layers and alterations of the cellular morphology were quantified. Other animals were allowed to give birth and the offspring were evaluated at 30 days of age for functional activity within the visual system using the quantified deoxyglucose method (Sokoloff).

The results of the histopathological studies and brain functional activity will be presented.

Supported by NIH Grant NS22766


Aspartame (Nutrasweet) is an artificial sweetening agent which is widely used by pregnant women. Previous reports have found developmental toxicity in rats only at very high doses. However, the guinea pig may be a better model for human toxicity than the rat. Virgin Duncan-Hartley guinea pigs were time mated in our laboratory and assigned to receive either 0, 250 or 500 mg aspartame/kg body weight in a sesame oil vehicle or the vehicle alone by mouth between day 1 of gestation and the day of birth. A non-treated control group was maintained. On the day of birth pups were weighed, sexed and allowed to remain with their natural mother.

At 15-16 days of age, odor aversion testing was carried out using LICI. At 30 days of age, brain glucose metabolic activity was quantified using the deoxyglucose method of Sokoloff et al. (J. Neurochem. 28: 897, 1977). Deficits in odor aversion testing were found to be correlated with altered patterns of brain glucose metabolism in the exposed offspring compared to the controls.

Supported by NIH grant NS22766

This study determined whether fetal substantia nigra (SN) grafts placed on the lateral ventricle contralateral to the lesion, produces improvement in the behavioral deficits produced by unilateral substantia nigra lesions. 10 Rates were injected with 8 ug/ul of 6-OHDA into the medial forebrain bundle (MBF). Apomorphine-induced rotation was determined 10, 20, 30 days after the lesion. On post-lesion day 34, 5 rats received fetal solid SN graft (E16) in the lateral ventricle contralateral to the lesion. Rotational behavior was determined at 10, 20, 30, 45 and 60 days after the graft and from then on each 15 days for the next 4 months. Rates were sacrificed and the tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) reactivity were determined. Grafted group performed less rotational behavior (RA) down to 0 days about 50% about 145 days after the graft, whereas the sham operated group increased rotation frequency.

We propose that humoral release of neuroactive substances produces a reduction in dopaminergic neurotransmission, causing them to return to a more normal level following the lesion. Spiperone-binding tests are currently underway in our laboratory to this suggestion.


Lesion of the nigrostriatal dopaminergic (DA) pathway induces a variety of morphological and functional changes in the denervated striatum, among others DA receptor upregulation, an increase of enkephalin (ENK) synthesis and content, of neuropeptide Y (NPY) content and following neonatal lesion- serotonergic (5HT) sprouting. The influence of DA-enriched neurons on these functional changes was studied.

Grafts were implanted into the denervated striatum in neonatal (PD6 or adult rat, 4 days after the unilateral destruction of the nigrostriatal DA pathway. Implanted DA-enriched neurons were stained with fluorescent markers and the corresponding animal was sacrificed 4 months after grafting. DA receptor hypersensitivity -as judged by apomorphine-induced rotation- in the striatum was assessed by comparing the rotation induced by apomorphine before and after grafting. The results suggest that an unequal influence of the grafts on the different target system in the striatum.


Our previous evaluation of neostriatal grafts placed in the kainic acid induced neostriatal lesion (KAIN) of adult rats indicated that minimal connectivity existed between the graft and the host brain at 2 months post-transplantation (Brain Res., 425: 34-44,1987). The purpose of the present study, performed at 6-8 months post-transplantation, was to determine if connectivity had changed. Five days after kainic acid lesion were made, cell-neostriatal primordia were injected into the lesion site. Eight animals with grafts received unilateral injections of lectin-inbound horseradish peroxidase into the ipsilateral substantia nigra. Tetrabromodeoxyuridine labelled neurons were used. Likewise, anterograde axonal label was absent within the intact NS and the lesion site adjacent to the transplant. Anatomical and functional studies show the possibility of connectivity occurs between lesioning agents and will be discussed.
CO-TRANSPLANTS: EFFECT OF FETAL STRIATAL TISSUE ON FETAL DOPAMINERGIC NEURONS.

The results of these experiments suggest that fetal striatal tissue may play a role in regulating the function of fetal dopaminergic neurons. This is supported by the observation that fetal striatal tissue caused a significant increase in DA release from fetal dopaminergic neurons, which was blocked by the D2 antagonist, sulpiride. These findings suggest that fetal striatal tissue may provide a neurotrophic influence on fetal dopaminergic neurons.

5-HT-immunoreactive cells were often seen. Numerous TH-positive cells were seen in the transplants. In addition, 5-HT-immunoreactive cells were often seen. Laminin-positive structures, presumably blood vessels, were widely distributed in the region of the striatal cell suspension were robust with extensive arborization and extended processes. Moreover, several TH-positive fibers extended processes through the ependymal wall of the ventricle and innervated the adjacent DA-denervated striatum.

Tyrosine hydroxylase immunocytochemical analysis of brain slices containing the co-grafts revealed that TH-positive grafts were located both within the nigral graft and also in the adjacent region in which the striatal cell suspensions were located. TH-positive cells located in the region of the striatal cell suspension were robust with extensive arborization and extended processes. Moreover, several TH-positive fibers extended processes through the ependymal wall of the ventricle and innervated the adjacent DA-denervated striatum.

Laminin-positive structures, presumably blood vessels, were widely distributed in the region of the striatal cell suspension. Male Sprague-Dawley rats were allowed to survive from 3 to 5 months before being used for experiments.


The development of human hippocampal transplants was studied using electrophysiological and anatomical methods. Transplants were grafted into the parietal operculum of adult rats and their development was monitored over a period of 10 weeks. The results showed that the hippocampal transplants developed normal electrophysiological properties and structural features similar to those of normal hippocampus. The findings suggest that human hippocampal transplants can be used as a model for studying the development of the human hippocampus and may have potential applications in the treatment of neurological disorders.
ONTotent of excitatory processes in the rat hippocampus region.

Dept. of Neurology, Univ. of Virginia Medical Center, Charlottesville, VA 22908.

Although some studies have compared hippocampal slices from young versus adult rats, a systematic in vivo characterization of the ontogeny of electrophysiologic responses in this structure has not yet been done. This and the accompanying study report responses obtained from rats of postnatal (PN) ages 7-65 days. Under urethane anesthesia, stimulating electrodes were placed in one CA3 region and a second recording electrode in the contralateral CA1 region. Stimulus intensity was varied to generate input-output curves for CA1 population spikes (PS). In younger animals, PS were broader, smaller in amplitude, and required higher intensity stimuli. However, after PN 14, excitability (measured by voltage required for half maximal PS) and PS duration (with an amplitude one-half peak for maximum amplitude) were not different from adult values. In contrast, maximum amplitude PS did not attain adult levels until PN 33. Conduction velocities (measured from time of stimulation to peak of evoked PS) steadily increased from PN 7 to PN 65. Thus, many, but not all, aspects of excitatory responses in CA1 hippocampal region are mature by PN 14.

Ontogeny of paired-pulse inhibition in the rat hippocampus region.

G. E. W. Lothman and H. B. Michaelson
Laboratory of Neurophysiology and Neuroepithelial Research, Brugmann and Erasme Hospitals, Université Libre de Bruxelles, Brussels, B-1070, Belgium.

We are examining the development of one measure of neuronal excitability, paired-pulse synaptic facilitation, in the rodent hippocampus, area CA1. Hippocampal slices are prepared from 2-4, 6, 8, 10, 13, 15, and >60 day rats, and maintained using standard slice procedures (2mM Ca2/Mm Mg). At least 4 animals and 8 slices have been sampled at each age. Stimulating (30um concentric bipolar) and recording electrodes (1-2mhm) are placed, on-line, in the middle of the apical dendritic field of CA1, in order to record the evoked synaptic field potential. Homosynaptically paired pulses (conditioned pulse = 40-60% max. field EPSP) are recorded across a range of interpulse intervals (10-5000ms), delivered at 0.33Hz. We are comparing the initial slopes of the conditioning and test pulse field EPSPs, as well as longer latency events, both within and across ages. Preliminary analyses demonstrate the presence of PPF at the earliest day tested, PN (e.g. 2.58% @ 20ms IPI; 16.98% @ 60ms; 12.98% @ 120ms; -9.97% @ 500ms), with gradual increases peaking in the 15 day slices (120% @ 20ms; 134.58% @ 60ms; 86.73% @ 120ms; 23.48% @ 500ms), with a slight decline in the >60 day group (87.8% @ 20ms; 96.33% @ 60ms; 63.25% @ 120ms; 18.22% @ 500ms). Excitatory processes appear to develop before inhibition, in CA1. Therefore the decreased level of PPF during the early postnatal age provides a margin of safety for the developing hippocampus. Supported by EPA, NIH, ONR.

Peptidergic neurons in the human hippocampus during development and aging.

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The distribution of Neuropeptide Y (NPY), Neurontensin (NT) Somatostatin (SOM), Cholecystokinin (CCK), and Substance P (SP) neurons is studied in the human hippocampal formation using immunocytochemistry of Sternberger. CCK and SP interneurons are mainly represented in the hilus, CA3, and CA2. NPY and SOM are more concentrated in CA1, subiculum and entorhinal cortex. Several interstitial NPY, SP, and SOM neurons are present in the angular bundle. Before three years of age, transient immunoreactivity is present in some neurons. These transient neurons are CCK interneurons in the entorhinal cortex, NT pyramidal neurons in the subiculum and CA2 and granule neurons in the area dentata. Although the density of NPY neurons decreases, the total number of these neurons is well established at birth, stays stable until at least the age of 42 and begins to decrease at the age of 60. Similarly the total number of SP neurons in the hilus region stays stable from birth until the age of 42. Supported by Belgium FRSM 3.4023.86 and PMBE 86.
LIMBIC SYSTEM I

354.11 BEHAVIORAL MATURATION AND PROTRACTED POSTNATAL PROLIFERATION OF PROGENITORS FOR GLUCOCORTICOID-SENSITIVE MOTONEURONS IN THE GOLGI CELL CIRCUIT OF THE PIG HYPOTHALAMUS. D. P. Webster and H.-P. Lipp. Institute of Anatomy, University of Zürich, CH-8091 Zürich, Switzerland.

Behavioral maturation may depend on protracted differentiation of neural circuitry long after the differentiation of sensori-motor systems (Flecksig's rule). The evidence for this rule is based on myelination cycles and protracted postnuclear growth of cell bodies. However, clear changes in the direction of axonal terminals have never been observed thus far.

Fifty-six guinea pigs, precociously born animals, were studied (6 per age group) at the age of 5, 10, 20, 40, 80, 160, and 320 days. Behavioral tests measured activity in a novel environment, and responses to ethical shock in a shuttle-box. The area of a plexus formed by recurrent mossy fibers below and on the granule cell layer was determined using means of multiunit image analysis. These mossy fiber contacts proliferate till the age of 40 days, and thereafter in some but not all animals. The postnuclear proliferation (which may reflect mossy synapses overgrowing inhibitory interneurons) was coincident with an increase of shock levels required to elicit behavior. Progress toward finding the genes responsible for these were found for the latency to start locomotion in a novel environment. So, Flecksig's rule applies to mossy fibers, too, and its significance may have specific behavioral consequences. Supp. by SNF 3.060-0.84.

THURSDAY AM

357 | SYMPOSIUM/WORKSHOP


Molecular genetic studies have elucidated the chromosomal location of a number of genes causing inherited neurologic and psychiatric diseases. For several of these diseases, new genes important in the function of the nervous system have been identified. This symposium will focus on genetic strategies used to find and characterize disease genes and their expression patterns. Examples of this approach, including transgenic mice, will be discussed. The symposium will provide an overview of current progress in identifying genes associated with diseases of the nervous system. Progress toward finding the genes responsible for diseases will be presented.


Tremblay will present evidence that synaptic noise during spontaneous release and physiological explanations for these gradients. The results of Tremblay and Robitaille indicate that synaptic noise arises from local and regional homogeneity of the MNJ, and that the MEPP amplitudes produced by the distal portion of the nerve terminal are smaller. Weitzman and others have demonstrated that in the frog NMJ, there are variations in the number of physiologically participating synapses (n) during presynaptic inhibition and long term facilitation. These results suggest that the large number of release sites may be the substrate for such plasticity. Henneman's results suggest that the lack of correlation between the single synapse (the frog NMJ) and the whole nerve terminal may be due to the different synapses for each fiber in the brainstem.

Supp. by SNF 3.081-0.84.

CELL LINEAGE AND DETERMINATION II


The division patterns of the cells that give rise to the embryonic peripheral nervous system (PNS) were studied using the thymidine analog BrdU. This method provides a very useful means of analyzing mutations that affect PNS development. For example, in embryos that are homozygous for mutations in the achaete-scute complex (as-C), the PNS is reduced in that as-C genes and are absent (Dawley-Chaomee, Ch. and Ghysen, A., Genes & Dev. 1: 297, 1987). These results could either be related to defects in the precursor cells or they could disrupt the normal proliferation of their progeny. By labeling mutant embryos with BrdU it was possible to distinguish between these possibilities by showing that the precursor cells for the missing sensory organs are either absent or fail to divide. The evidence suggests that periodic cell divisions may affect the development of the embryonic PNS of Drosophila.


Engrailed (en) has been identified in Drosophila as an important developmental gene involved in the control of segmentation. A chick homologous to the Drosophila en gene was identified using a cDNA library from the 2-cell stage that hybridizes to mRNA of the 4-cell stage embryo. A 35S-labeled RNA probe (CS, KB) in Northern analyses, the probe hybridized to RNA from day 4 chick embryo (5.0, 3.5, 2.2 KB) [DD,CS,KB]. A 1.2Kb fragment of the 35S-labeled RNA probe (CS, KB) in Northern analyses, the probe hybridized to RNA from day 4 chick embryo (5.0, 3.5, 2.2 KB) [DD,CS,KB]. A 1.2Kb fragment of the 35S-labeled RNA probe (CS, KB) in Northern analyses, the probe hybridized to RNA from day 4 chick embryo (5.0, 3.5, 2.2 KB) [DD,CS,KB].
Diverse neuronal types arise from a single progenitor in chick optic tectum. Studies using a retroviral sarcoma virus as a lineage marker. C. Walsh, C. L. Cepko. Depts. of Anatomy & Neurobiology, and Biological Chemistry, Washington Univ. School of Medicine, St. Louis, MO 63110.

We are using recombinant retroviruses to trace cell lineage in the chicken optic tectum. These replication-defective vectors insert the E. coli β-galactosidase (lacz) gene into the genome of an infected cell; the cell's lacz activity permits histochemical identification of clones derived from the infected cell. This vector was used to trace clones of neurons and glial cells in the optic tectum. The tectum was divided into 25-200µm layers, separated laterally by 25-200µm. Larger groups of labelled neurons were found in the thalamus. Labelled cells were of intermediate morphology and contained both neuronal and glial markers.

Thus, a common progenitor apparently produces all four major classes of neurons and glia, which may share common properties and mechanisms of differentiation.

Little is known about the factors that regulate neuronal production during the period of postnatal development in the rat retina. We investigated the effects of dibutyryl cyclic AMP (cAMP) on the proliferation of retinal cells in vitro. Our results indicate that cAMP stimulates the proliferation of postnatal retinal cells.


We have investigated the three-dimensional distribution of peptides in developing brain using immunohistochemistry. Our results suggest that the distribution of peptides is influenced by the developmental stage of the brain.

THURSDAY AM

CELL LINEAGE AND DETERMINATION II

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359.11 INDUCTION OF MITOMETAR REST AND MORPHOLOGIC DIFFERENTIATION OF NEUROBLASTS BY NEOCARZINOSTATIN: CYTOCRITICAL AND THERAPEUTIC IMPLICATIONS. N. P. Schor, Div. of Child Neurology, Children's Hospital, Pittsburgh, PA 15213.

Neocarzinostatin is a chemotherapeutic agent that affects the proliferation and differentiation of neuroblast cells. Our results suggest that neocarzinostatin induces cytocritically directed differentiation of these cells.


We have developed a method for double-labeling immunohistochemistry to categorize glial cells in sections of rat brain. Our results suggest that this method can be used to study the morphology and distribution of glial cells.

SYNAPTogenesis III

360.1 VIDEO MICROSCOPY OF FIRST CONTACTS IN CAI HIPPOCAMPAL CELL CULTURES. M. W. Cooper* and S. J. Smith (SPION P. Forscher). Section of Molecular Neurobiology, Yale University School of Medicine. 333 Cedar St., New Haven, CT 06510.

We have developed video microscopy to observe neurons establishing their first contacts in newborn rat hippocampal cell cultures. Our results suggest that this method can be used to study the formation of neuronal contacts.

360.2 GRADUALLY LOSS OF MULTIPLE INNERVATION VISIBLE WITH A SIMPLE CONFOCAL MICROSCOPE. T. L. Schaal, J. J. Berliner, and J. L. Sunderland. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have developed a method for visualizing multiple innervation in developing neurons using a simple confocal microscope. Our results suggest that this method can be used to study the distribution of neuronal contacts.
360.3 ELIMINATION AND ELABORATION OF PRE- AND POSTSYNAPTIC SITES DURING NEUROMUSCULAR JUNCTION DEVELOPMENT. B.J. Balice-Gordon and J.W. Littmann, Department of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110

We have studied the sequence of pre- and postsynaptic events during the elimination of multiple innervation at developing neuromuscular junctions in mouse embryos. Using 4D2-APP, a lectin label mouse neurons and a non-blocking dose of fluorescently tagged alpha-bungarotoxin to label postsynaptic acetylcholine receptors, the same junctions were repeatedly studied at short intervals during the first postnatal month. At multiple innervated junctions, some nerve terminal branches and the postsynaptic acetylcholine receptors underlying them are rapidly eliminated, often in less than one hour. The aggregate sequence of events at the same junction is terminal and receptor sites are gradually elaborated. This elaboration continues until the end of the first postnatal month, resulting in junctions that gradually become more complex. After this time, the configuration of both nerve terminals and receptors is remarkably stable, enabling by simple expansion as muscle fibers grow.

Thus elimination and elaboration of synaptic sites are concurrent events. Interestingly, at multiple innervated junctions the remaining axon does not elaborate terminals at sites vacated by eliminated terminals. Eliminated terminal and receptor sites therefore represent permanent alterations in the arborization pattern of the endplate.

These results suggest that axons do not compete for occupation of the same postsynaptic sites, and that competition alters only nerve terminals but not postsynaptic acetylcholine receptors.

360.5 LOCAL ACCUMULATION OF ACETYLCHOLINE RECEPTORS IS INSUFFICIENT TO INDUCE CLUSTERING. J. Stoffler and S.E. Fratz, Dept. Physiology and Biophysics, UC Irvine, Irvine, CA 92717

Acetylcholine receptors (AChRs) aggregate at the developing neuromuscular junction by mechanisms not fully understood, but at least in part via lateral migration. We have developed an in vitro model system - cultured Xenopus muscle cells exposed to external electric fields - to aggregate the AChR. The aggregated AChR is concentrated at the cathode-facing pole, and can be isolated, aggregated, and concentrated in agarose gels. The results indicate that the aggregation involves other molecule(s), a voltage-sensitive mechanism, or both. We need further studies on secreted molecules or other factors to account for the observed voltage-sensitive mechanism, or both.

Treatment of myoball cultures with neuraminidase changes the charge on the cell surfaces, which has been reported to reverse the direction of electrophoretic migration for AChRs and concanavalin A binding sites (Orida and Pocock, 1981). We have found that the field-induced AChR concentration switches to the anode-facing pole after field termination, but decreases at the anode-facing pole - clear evidence that elevated AChR density alone does not initiate post-synaptic aggregation.

Findings suggest that axons do not compete for occupation of the same postsynaptic sites, and that competition alters only nerve terminals but not postsynaptic acetylcholine receptors.
360.11 GLUTAMATE RELEASED FROM ENTORHINAL CORTEX AXONS INHIBITS DENDRITIC OUTGROWTH AND PROMOTES SYNAPTOGENESIS IN HIPPOCAMPAL NEURONS.

A culture system of embryonic rat entorhinal cortex explants and isolated hippocampal neurons was used as a model of hippocampal development to determine that endogenously-released neurotransmitters can shape neuronal circuitry. Hippocampal neuron dendrites were visualized by MAP2 immunocytochemistry on the background of nonsynaptic entorhinal axons; little dendritic outgrowth occurred in neurons on the axon bed. Since glutamate is the major excitatory neurotransmitter in isolated hippocampal neurons (Matson et al., J. Neurosci. 8:2087, 1988), we hypothesized that glutamate, released from entorhinal axons, might be suppressing dendritic outgrowth. HPLC analysis of culture medium showed activity- and calcium-dependent release of glutamate from explants. The glutamate receptor antagonist di-glu tamylglycine (DGG) significantly increased dendritic outgrowth in hippocampal neurons on the axon bed. Tetrodotoxin, elevated extracellular Mg2+, or removal of essential cell bodies also enhanced dendritic outgrowth; apparently ongoing activity in entorhinal neurons and associated glutamate release was responsible for outgrowth inhibition. Both the geometry and synaptic organization of a neuron determine its information coding capabilities. We therefore examined synaptic correlates of glutamate's effects at the light (Timm method and protein III staining) and ultrastructural levels. In control cultures synaptic specializations associated with the axons and dendrites of hippocampal neurons were abundant and well differentiated containing numerous small (30-50 nm) vesicles. In contrast, synapses in DGG-treated cultures were reduced in number and were poorly developed with relatively few, large (>100 nm) and irregular-shaped vesicles. The mechanism by which glutamate inhibits outgrowth and promotes synaptogenesis apparently involves sustained elevations in intracellular calcium; DGG reduced intracellular calcium levels in hippocampal neurons on the axon bed. Tetrodotoxin, elevated extracellular Mg2+, or removal of essential cell bodies also enhanced dendritic outgrowth; apparently ongoing activity in entorhinal neurons and associated glutamate release was responsible for outgrowth inhibition. Both the geometry and synaptic organization of a neuron determine its information coding capabilities. We therefore examined synaptic correlates of glutamate's effects at the light (Timm method and protein III staining) and ultrastructural levels. In control cultures synaptic specializations associated with the axons and dendrites of hippocampal neurons were abundant and well differentiated containing numerous small (30-50 nm) vesicles. In contrast, synapses in DGG-treated cultures were reduced in number and were poorly developed with relatively few, large (>100 nm) and irregular-shaped vesicles. The mechanism by which glutamate inhibits outgrowth and promotes synaptogenesis apparently involves sustained elevations in intracellular calcium; DGG reduced intracellular calcium levels in hippocampal neurons on the axon bed. Tetrodotoxin, elevated extracellular Mg2+, or removal of essential cell bodies also enhanced dendritic outgrowth; apparently ongoing activity in entorhinal neurons and associated glutamate release was responsible for outgrowth inhibition.
361.3 The Monkey Homolog of the β-Amyloid Precursor Protein of Alzheimer's Disease: Identification of Novel Forms of the Protein. H. Berman-Podlinsky, B. Tolan and D. Selke (SPON: G. Strichartz). Harvard Med. Sch., Brigham and Women's Hosp., and Boston Univ. of Healthcare, Boston, MA. Alzheimer's disease is always accompanied by the deposition of extracellular filamentous composed of the β-amyloid protein (8-42). Studies of aged monkeys during normal brain aging in humans and lower primates. We have shown that amyloid in aged monkey cortex contains βAP anti- genically indistinguishable from that in AD. Thus, aging monkeys may prove useful for study of the βAP in human brains. The different newly synthesized βAPP's may be discussed with respect to a possible precursor-product relationship in AD.

361.5 The Biological Activity of Alzheimer's Amyloid β-Protein. B. A. Yankner, L. Villa-Komaroff and R. L. Noveck (Department of Neuroscience and Genetics, The Children's Hospital, Boston, MA 02115). The rat phagocytosis cell line PC12 differentiate into a neuronal cell type in response to nerve growth factor (NGF). Northern blot analysis demonstrated that the amyloid precursor RNA is expressed constitutively in PC12 cells and inducible about 100-fold by treatment with NGF. cDNAs corresponding to the amyloid precursor or a fragment containing the amyloid β protein precursor RNA transfected into PC12 cells by calcium phosphate method. Several clones were isolated that overexpressed either amyloid β protein or amyloid precursor RNA from the transfected cDNAs. Clones overexpressing the amyloid β protein exhibited a significantly increased neurite outgrowth in response to NGF. Clones overexpressing the amyloid precursor also exhibited an increased neurite outgrowth but relatively less than cells expressing the amyloid precursor RNA. Conditioned medium from transfected cells greatly potentiated neurite outgrowth when added to untreated PC12 cells or cultured dorsal root ganglion neurons. This study suggests that amyloid β protein may play a role in neuronal differentiation.

361.6 Development of a Genetically Engineered Cellular Model for the Amyloids of Alzheimer's Disease. S. S. Tamaka, W. G. Chou, R. A. Malhotra, and C. A. Mucke (SPON: G. Hauser). Univ. Rochester Cancer Ctr., Rochester, NY 14621 and Harvard Med. Sch., Massachusetts General Hosp., Boston, MA 02114; Neuman Res. Ctr., McMaster Hosp., Burlington, MA 02178. The Aβ domain of the amyloid precursor protein (APP) cloned directly from Alzheimer's disease (AD) brain mRNA has the same structure as from non-AD sources (Zain, et al., PNAS 85:9299, 1988). Local processing of the precursor may be a critical event in the formation of amyloid plaques. Therefore, we prepared genetically transformed cells that express the Aβ domain. Transfection was carried out with synthetic vectors, originally derived from SV40, that were linked to the 1.1 kb DNA Eco RI fragment that contains the Aβ region. Successful transformation was confirmed by Northern blots and in situ hybridizations. Cells were immunostained with monoclonal antibodies to the Aβ peptide. Transfectants can be used to examine the consequences of amyloid over-production and to define conditions that interrupt this process. Supported by AG02126, CA11198, CA36432, American Health Assistance Foundation and Familial A.D. Res. Foundation.

361.8 Tau Protein in Alzheimer's Disease, Aged Rats and Systemic Organisms. S.C. Papazianouzokos and L. I. Binder, Dept. of Pathology, Univ. of Texas Med. Sch., Houston, TX 77225 and Deps. of Cell Biol. and Neurology, Univ. of Alabama, Birmingham, AL 35294. We have shown that in Alzheimer's disease (AD) excessive tau is transported to the cytoplasm of neurons and astrocytes. We have also shown (Papazianouzokos, S. C. and Binder, L. I., Cell Motil. Cytoskel. 8(10), 1987) by using two mono- and polyclonal antibodies against Tau-1 and Tau-2, that phosphorylated Tau-1 epitope and the Tau-2 epitope are present on microtubules and ribosomes in the putamendolencecom partment of neuron and in astrocytes in order to gain further insight into the physiologic functions of tau and the etiopathogenesis of AD, we have examined the tau immunoactivity in almost every systemic organ and the CNS of several Sprague-Dawley rats aged 6 weeks to 25 months old. Among the systemic organs, the most detectable in plasma cells, the islets of Langerhans and the renal medullary collecting duct epithelium. In the CNS, the tau-immunoreactivity was determined in the compartment of neurons and astrocytes without prior dephosphorylation of sections up to the age of 3 months, but in older rats tau immunoactivity was eliminated in the Tau-1 epitope, and thus detectable without prior dephosphorylation of sections, appeared, besides axons, in protoplasmic astrocytes. These astrocytes were most numerous in cortical layer IV, the inferior colliculi, the granule cell layer of cerebellum, the CA3 region of hippocampus and the dorsal half of the lumbar spinal cord. Consequences of the findings suggest that a disturbance of a secretory process in which tau may play a role may be involved in AD. These findings suggest that a disturbance of a secretory process in which tau may play a role may be involved in the pathogenesis of aging and AD.

Several diseases of the nervous system, such as Alzheimer's, Pick, Parkinson and amiotic lateral sclerosis are characterized by the presence of intraneuronal inclusions whose composition and mode of formation remains unclear. We have induced the formation of intracytoplasmic neuronal inclusions exposing rat dorsal root ganglia to the antibiotic drug xylocain-sarinobin (STX(6-8)-M), for 7 days. The inclusions are round, approximately 10 microns in size and markedly eosinophilic. Ultrastructurally they are composed of packed filaments mixed with trapped cytoplasmic organelles. The inclusion immunoreact with antibodies to Ubiquitin, Tau proteins and phosphorylated epitopes of the 200 kDa neurofilament subunit, but not with antibodies to actin and Heat Shock Proteins 70 and 28. Antitubulin antibodies react only with those inclusions. The experimental inclusions we have obtained may provide a model to study the mode of formation of those neuronal inclusions that form in the course of human neurodegenerative conditions and that contain Ubiquitin, Tau proteins and phosphorylated epitopes of neurofilaments.

 Supported by NIH Grants NS14503 and AG00795.


Alz50 is a monoclonal antibody directed against an antigen that is present at higher levels in Alzheimer versus control brain (Wolozin et al., SCIENCE 1986). Recently, we have shown that the antibody reacts with tau in all of its heterogeneous forms (Fukada et al., EUROSCI LETT 1988). We therefore sought the epitope in tau with which the antibody reacts. Alz50 does not react with any of the epitopes that specifically synthesize tau, thus suggesting the antibody is directed against a post-translational modification or a conformation not achieved in the bacterial cell. Bovine tau was digested with cyanogen bromide (CNBr) and the HPLC-separated fragments tested for Alz50-reactivity. The reactive fragments elute over a broad range with a late retention time relative to other CNBr fragments. Tubes with Alz50-reactivity contained two tau peptides, both of which derived from the carboxy half of the molecule. The carboxy half of tau is likely to contain the microtubule binding domain. One of the peptides is likely to contain the Alz50 epitope, which may represent a site on tau that is involved in the early stages of neurofibrillar tangle formation.
362.3 FUNCTIONAL ORGANIZATION OF VISUAL AREA 18 OF MACAQUE AS REVEALED BY VOLTAGE-SENSITIVE DYES AND INDEPENDENT INTRACRANIAL SIGNALS. D.Y. Trau, R.D. Frostig, E.E. Liaw, A. Grinvald. Laboratory of Neurobiology, The Rockefeller University, NY, 10021 and IBM Division, Yorktown Heights, NY 10598.

Using a high S/N CCD camera, we have extended the spatial resolution of the imaging of intracranial microelectrode potentials, which are a measure of electrical activity that can be used to infer functional areas of the visual cortex. The method has been applied to study the functional organization of the visual cortex of the macaque, as revealed by voltage-sensitive dyes (VSDs) and independent intracranial signals. The technique allows for the mapping of functional areas with high spatial resolution and sensitivity, providing insights into the organization of the visual cortex and its responses to various stimuli.


We have used voltage-sensitive dyes in conjunction with focal electrical stimulation to map connections between visual areas in the anesthetized squirrel monkey. DC evoked V1 activation was studied with a styryl dye (RH795, courtesy A. Grinvald and R. Hildesheim), and imaged onto a 10 x 10 photodetector array. Area V1 was stimulated with a brief train of pulses passed through a tungsten microelectrode. Large responses were seen with current pulses as low as 100 nA and 0.2 ms (histoplasmin).

Within V1, local responses extended several mm. from the stimulation site; the full-width at half-height was 1-2 mm. Responses in the periphery of this zone began 4 sec or more after those in the center, which presumably reflects conduction and/or synaptic delays.

In two hemispheres, responses were seen at the expected topographic location in V2. These were well separated from the VI stimulation site and began with a delay of 6-8 msec. The region of activation in these instances included all three cytochrome-oxidase-stained (thick, thin and inter-stripe). Thus, this paradigm should be valuable for probing the topographic (and probably compartmental) organization of cortical visual areas. Supported by the Office of Naval Research.


We measured the spatial frequency (SF) tuning of cells at retinal and cortical levels along tangential fibers through the supragranular layers of the monkey's striate cortex, and correlated the recording sites with the cytochrome oxidase histochemistry. We found that SF tuning curves have three main properties in the striate cortical organization:

1. There is a periodic anatomical arrangement of cells tuned to different SFs, with a periodicity of 0.6-0.7 mm.
2. Are just two populations of cells, low SF and high SF, at a given eccentricity, or is there a continuum? There is a continuum, with most tuned to the intermediate SFs, forming a non-uniform distribution.
3. What is the relation between physiological SF organization and the cytochrome oxidase histochemistry? The preferred SF in successive loci changes systematically: it gradually rises and falls. The cycle repeats with a period of about 0.6-0.7 mm.

362.6 FUNCTIONAL ORGANIZATION OF THE BLOB CELLS IN THE MONKEY S'S STRIATE CORTEX. Charles R. Michael, Dept. of Cellular and Molecular Biology, The School of Medicine, New Haven, CT. 06510.

Closely spaced, perpendicular tungsten electrode tracks were made through the cytochrome oxidase blobs of the supragranular layers of the monkey's striate cortex to examine the relationship of the various cell types to one another. Double opponent color cells seemed to be concentrated in the centers of the blobs; broad band units were found primarily at the edges and in between, were Type II and modified Type II neurons. Double opponent cells were maximally excited by presenting a color in the center while simultaneously illuminating the surround with the complementary color. Spectral sensitivity curves revealed that these color surrounds were organized in an opponent color manner. Some of these cells, particularly in layer 2, did not respond to color; modified Type II cells never discharged to annular stimuli. Their surrounds had broad band spectral sensitivities, consistent with a response to large spots or two-colored stimuli. Type II cells were often seen in layer 3 while modified Type II neurons were more prevalent in layer 2. Supported by NIH Grant EY 00568.

362.7 A COMPUTER SIMULATION OF CORTICAL ORIENTATION SELECTIVITY IN THE CAT VISUAL SYSTEM. U. J. Wehrmeier and C. Koch, Division of Biology, 216-76, Caltech, Pasadena, CA 91125.

The manifestation of orientation tuned responses in cells of visual cortex has been addressed by models utilizing excitatory feedforward connections (Hubel & Wiesel,1961). More recently, one of us has proposed an excitable model of orientation selectivity, combining aspects from all these models (Ferster and Koch, 1987). We have implemented a computer simulation of a 2x2 degree patch of visual angle in the retina, its projection to LGN, and with different peaks were found to be continuously and smoothly distributed across a module.

362.8 INHIBITORY AND EXCITATORY CONTRIBUTIONS TO ORIENTATION TUNING IN THE CAT'S STRIATE CORTEX. F. Würgler* and U.T. Eysel, Institute of Physiology, Ruhr-Universität Bochum, D-4600 Bochum, F.R.G.

Orientation specificity in the visual cortex can be generated by excitatory convergence of OFF-driven intrinsic connections, intrinsic circuitry and independent intracranial inhibition (Ferster, D. & Koch, C., TINS, 10:487, 1987). Our study utilized local lateral inactivation of cortical tissue by GABA microiontophoresis to investigate the influence of intracortical mechanisms. Recordings were made from cells in layers III-VI near area centrais in the striate cortex of anesthetized cats while stimulating with moving light bars. GABA microiontophoresis showed that cells at distances relative to the recorded cell and changes of the response characteristics were quantified. Orientation tuning was significantly reduced for S and C-center cells upon GABA inhibition contributing to orientation selectivity was demonstrated predominately by local inactivation at distances of about 0.5 mm. Such loss of lateral inhibition in layer IV was prevented by GABA inhibition in the generation of orientation tuning in addition to the direct CGL input. Lateral excitatory effects were most frequently affected by inactivation at distances of about 1.0 mm. To determine the interdependence of direction and orientation tuning correlation coefficients between the strength of both specificities were calculated and only a weak coupling was found. This supports our previous evidence that different mechanisms underlie direction and orientation specificity (Eysel et al., J.Physiol. 399:657, 1988). The results further suggest that both excitatory and inhibitory connections from laterally displaced cells can contribute to the generation of orientation specificity.
362.9


We have developed a model of simple cell receptive field properties (RFs) based on the following assumptions: linearity of summation in 2D space and time, biophysical time course of inputs and Hubel-Wiesel geometry of LGN inputs configured as push-pull (class B) amplifiers. By push-pull, we mean that all points in the RF are excited by inputs of one center sign (push) and inhibited by inputs of the opposite center sign (pull). We will show that this model accounts for the following aspects of RF function: a) structure in space-space-time b) orientation selectivity c) spatial summation d) spatial frequency selectivity e) cospatial distribution of spots to bright (dark) and ipps to dark (bright) stimuli f) optimal direction and velocity g) action on ganglion blocking by retinal APB and h) action of GABA blockers.

362.11

X- AND Y-LIKE RECEPTIVE FIELD PROPERTIES IN CAT AREAS 17 AND 18. C. Handsell* and D. Perger. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

In a recent abstract, Perster (Neurosci. Abstr., 13:1449) reported that X- and Y-mediated optic potentials, identified by their threshold stimulation of the optic nerves, are largely segregated between areas 17 and 18 of cat visual cortex. Does this apparent segregation give rise to differences in receptive fields of cells in the two areas? Movshon et al. (J. Physiol. 283:101) found that preferred spatial frequencies in area 17 were on the average 3-fold higher than those in area 18; this parallels the difference in preferred spatial frequencies of X and Y cells. We report two further distinctive features of receptive fields in area 17 and 18.

1) Y-cells are identified by their phase-independent, frequency-doubled response to stationary contrast-modulated gratings, which is most evident at high spatial frequencies. Spitzer and Hochstein (J. Neurophysiol. 53:1245) failed to find evidence for this type of nonlinear response in receptive fields of cells in the two areas! Movshon et al. (J. Physiol. 283:101) found that preferred spatial frequencies in area 17 were on the average 3-fold higher than those in area 18; this parallels the difference in preferred spatial frequencies of X and Y cells. We report two further distinctive features of receptive fields in area 17 and 18.

2) Y-cells have on average a 3-fold higher contrast sensitivity than X-cells (Troy, Vision Res. 27:1733). Paralleling this difference, field potentials evoked in area 18 by stationary contrast-modulated gratings have approximately 3-fold higher contrast sensitivity than field potentials recorded simultaneously in area 17.

These differences in receptive field properties support the apparent segregation of X and Y input into areas 17 and 18 and found intracellularly. Since with four independent methods, evidence for Y input to area 17 cannot be demonstrated, we suggest that the Y input to area 17 differs fundamentally from that in area 18, or is minimal in its extent.

362.12


An optimal stimulus presented within the receptive field of neurons in the cat visual cortex evokes a correlated oscillation of both the local field potential (LFP) and neural activity. These oscillations provide a mechanism by which local intracolumnar populations of neurons temporally coordinate their activity. To test this hypothesis we recorded both LFP and neural oscillations simultaneously across columns within a cortical layer, and within all layers of the same column using 4-16 closely-spaced electrodes (ISO-400 pm separation) in areas 17 and 18 of the cat visual cortex. The visual stimulation 1) that oscillations of both the MUA and LFP can occur in precise synchrony throughout all layers of the cortex, as well as independently in upper and lower layers, and 2) that the active population of cells engaged in the synchronous oscillations can extend from 300-1500 pm. Sharp boundaries of synchronous oscillatory activity occur at the junctions of columns having orientation preferences which differ by 60-90 degrees. The results support the hypothesis that neuronal oscillations provide a mechanism by which local populations of neurons synchronize their activity in response to specific sensory stimuli.

362.13


In the previous report local populations of neurons, within columnar boundaries, were shown to engage in stimulus-specific coherent oscillations throughout different cortical layers. This suggests that relations between common, but spatially separate, features in the visual field may be established by the synchronization of oscillatory responses in different parts of the visual cortex. To test this hypothesis we recorded multunit activity (MUA) and local field potential (LFP) responses simultaneously across columns in area 17 of the cat from multiple electrodes separated by 2-8 mm in the cortex while stimulating the respective receptive fields with moving light bars. Cross correlation and coherence analysis of the signals demonstrated that significant stimulus-dependent correlations of oscillatory responses occur between columns within the same area and between areas 17 and 18. The correlation of oscillations in the two areas varied from 0.3 to 0.7, suggesting that the oscillations occur in phase with a variation of phase lag of 3 ms. 

362.10


We estimated the shape of a cortical threshold curve to gain insight into the properties of a function that such a curve would predict. We measured responses in an OFF area of a simple cell that showed strong threshold nonlinearity when it was presented with an optimally oriented bar whose luminance was modulated randomly. We then characterized the dynamic behavior of the pathway from retina to cortex by calculating a family of functions that are related to Wiener kernels. The functions are used to describe linear transients in the neural response, and also nonlinear contributions that arise from interactions in time and intensity. Because cortical transformations are difficult to measure directly, we modeled the neural pathway as a cascade of a linear dynamic, a static nonlinear, and a second dynamic linear transformation (LNL model) in which only the linear transformations showed temporal filtering. We suggest that the first transformation should be a linear system. The only dependence of responses on the sequence of transformations in a system with alternating dynamic-linear and static-nonlinear stages facilitates testing model hypotheses about the sequence and nature of neural events. The resolution and completeness of the measurements allowed us to identify the first linear filter as lowpass and the second as more strongly highpass. A fourth-degree polynomial representation of the linearized static nonlinearity, which we believe to represent the threshold nonlinearity of the measured simple cell, suggests that the onset of action-potential generation is gradual. This gradual transformation is well represented by a squaring function for positive input signals, i.e., a "squared," which implies that a low order of nonlinearities will suffice for an accurate representation.

Our results suggest that (1) intracellularity, single cells carry a highly linear representation of image luminance; (2) cortical thresholds are gradual, which makes them ideal for nonlinear operations such as squaring, an operation that has been proposed for movement energy models; and (3) the action-potential generator itself may initiate the periodic process that we measure as the second linear filter. (Supported by EY00479, EY01339, NSERC (Canada), EY04711, and EY00250.)
363.1 MULTIPLE CALCIUM CURRENTS IN CULTURED EMBRYONIC AMPHIBIAN SPINAL NEURONS. M.S. Bahr. Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

As part of an analysis of the roles of ion channel activity in neural development, I have studied the Ca currents of cultured embryonic Xenopus neurones during the period of initial differentiation and neurite outgrowth. Cultures are prepared from nerve and muscle precursor cells isolated from the spinal cord of 4-6 day larval stage embryos. Ca currents in differentiating neurones are studied using conventional whole-cell patch-clamp techniques. Cells are grown on serum-coated glass coverslips to minimize neurite growth, and neurons with processes extending less than one soma diameter can be studied routinely. Ca currents show monotonous activation and rapid deactivation, indicating of good spatial control of membrane voltage.

Three components of Ca current can be separated based on kinetics and pharmacology. A fast transient component shows an activation threshold of -40 mV and activation and steady-state inactivation curves overlap between -40 and -30 mV. These transient currents can be blocked by 100-150 µM Ni. In the presence of Ni, currents showing incomplete slow inactivation (during 1 sec depolarizations) are recorded at voltages >-30 mV. The inactivating portion shows an activation threshold of -30 mV, and overlap of activation and inactivation curves (measured using 1 sec conditioning and test depolarizations) occur between -30 and -10 mV. Shifting the holding voltage to -40 mV exposes a non-activating inward current that can be activated at voltages >-10 mV. These components correspond to the T-, N- and L-type Ca currents that have been described in other excitable cells.

The resting potential of these neurons is between -50 and -60 mV, and the action potential threshold is about -25 mV. Thus two of the components of Ca current observed may mediate Ca entry during subthreshold voltage excursions.


Electrical and pharmacological properties of low-threshold Ca channels have been studied in neurones isolated acutely from adult rats using both intracellular perfusion and concentration-clamp techniques. In most cells a definite low-threshold inactivating component was found. The low-threshold component was activated at membrane depolarization to -60 mV from a holding potential level of -100 mV, and it inactivated exponentially in a potential dependent manner. The steady-state inactivation occurred at very negative membrane potentials, reaching 50% level at -90 mV. Low-threshold Ca channels were blocked in the order of La > Zn > Cd > Ni > Co and of flunarizine > nifedipine > niflidipine > nifedipine > 5-hydroxy-50 > diltiazem. Substitution of Ba for Ca reduced the current by 30-50% while substitution of Sr for Ca did not produce definite changes in current amplitude.


Differentiation of PC12 cells Induced by the presence of NGF for 10 days leads to a 10-fold increase in the density of Ba²⁺ current in the cell body and to a marked increase of the fraction of Ba²⁺ current which is reversibly blocked by ω-Conotoxin. Holding potentials negative to -40 mV recruit a current component which inactivates slowly (<50-100 ms) and completely. The single channel charge underlying this component of whole cell current is similar in its main conductance (18-22pS), activation range, and mean open time to the dihydropyridine (DHP) sensitive L-type calcium channel, but it can be distinguished from the L-type channel by its lack of sensitivity to the DHP agonist (+) 202-791, inactivation rate during test depolarizations, and more negative steady-state inactivation range.

363.4 VOLTAGE-DEPENDENT CALCIUM CONDUCTANCES AND MAMMARY BODY NEURONS AUTORYTHMICITY: AN IN VITRO STUDY. A. Alonso* (SPONSOR: A. Alonso). Physiol. Sci., Univ. of Chicago, Chicago, IL 60637

Voltage and current-clamp recordings from mammalian and pre-mammalian body neurones indicate the presence of three voltage-dependent Ca-conductances. In current-clamp studies, depolarization from a membrane potential of -80 mV, elicited typical low-threshold calcium spikes (LTS). The density of LTS was found to be low for a given holding potential (PP), which were especially clear after TTX. Depolarisations from a more positive potential (-50 mV) could evoke PPs in isolation from the LTS, as the conductance of PPs (PFS), which were close to zero at -50 mV. After TTX, the PPs always generated high threshold Ca-spikes. Under voltage clamp from a holding potential of -85 mV a typical transient inward Ca-currents were first observed at -65 mV reaching a maximum peak at approximately -40 mV. A second inward Ca-current with slower activation and inactivation kinetics and a higher activation voltage followed the transient ICa. Near -20 mV the voltage control was often lost as high threshold spikes were invariably evoked. These findings indicate that two different Ca-conductances are present in mammillary neurones. These provide the electroresponsiveness needed to support the pacemaker autorythmicity involved in the generation of theta rhythm in the limbic system. Supported by NHI grant NS13742 and a Fellowship to A.A. from the Ministerio de Educacion y Ciencia (Spain).


The Ca⁺⁺ current in acutely dissociated hippocampal CA1 pyramidal cells, activated rapidly (1-2 ms) on depolarizing the cell to potentials above -40 mV. The current then inactivated with a slow time constant that was characterized by the sum of 2 exponentials (time constants ~ 200 and 2000 ms) plus a constant offset. Both time constants decreased monotonically with increasing step potentials, suggestive of an exponential dependence on voltage for inactivation. However, when a protocol to disclose Ca⁺⁺ transient inactivation was used (i.e. a real inactivating prepulse (75) to varying potentials, followed by a step to a constant potential to assay the amount of remaining current), the time course of the decay of all three phases in the postpulse exhibited a minimum at the peak of the IV curve. The resulting U-shaped dependence of postpulse amplitude on voltage suggested a Ca⁺⁺-induced inactivation. However, contrary to the expectations of this form of inactivation, when the fast Ca⁺⁺-buffered BAPTA, was added in the intracellular medium and Ba⁺⁺ extracellularly, the U-shape of the inactivation curve persisted. Moreover, under conditions where all current passing through the Ca⁺⁺ current was borne by Na⁺, the U-shape of the inactivation curve still persisted.

Supported by NIH grant NS 24519 to R.K.S. Wong, Columbia University.

363.6 RELATIONSHIP BETWEEN CALCIUM CURRENTS, ICa, AND INTRACELLULAR CALCIUM CONCENTRATION, [Ca²⁺]i, IN SENSORY NEURONS. Stanley A. Tae博 and Richard J. Miller Dept. of Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Simultaneous whole cell patch clamp and four-2 microfluorometric recordings of ICa and [Ca²⁺]i were made from sensory neurones grown in primary culture from the dorsal root ganglion of the rat. Cells were held at -80 mV and depolarized to 0 mV eliciting a ICa that resulted in a [Ca²⁺]i transient which lasted long after the cell had repolarized and the current stopped. When the cell was depolarized for different test pulse durations the relation between the integrated ICa and the peak of [Ca²⁺]i was found.

The Ca²⁺ current is acutely dissociated hippocampal CA1 pyramidal cells, activated rapidly (1-2 ms) on depolarizing the cell to potentials above -40 mV. The current then inactivated with a slow time constant that was characterized by the sum of 2 exponentials (time constants ~ 200 and 2000 ms) plus a constant offset. Both time constants decreased monotonically with increasing step potentials, suggestive of an exponential dependence on voltage for inactivation. However, when a protocol to disclose Ca⁺⁺ transient inactivation was used (i.e. a real inactivating prepulse (75) to varying potentials, followed by a step to a constant potential to assay the amount of remaining current), the time course of the decay of all three phases in the postpulse exhibited a minimum at the peak of the IV curve. The resulting U-shaped dependence of postpulse amplitude on voltage suggested a Ca⁺⁺-induced inactivation. However, contrary to the expectations of this form of inactivation, when the fast Ca⁺⁺-buffered BAPTA, was added in the intracellular medium and Ba⁺⁺ extracellularly, the U-shape of the inactivation curve persisted. Moreover, under conditions where all current passing through the Ca⁺⁺ current was borne by Na⁺, the U-shape of the inactivation curve still persisted.

Supported by NIH grant NS 24519 to R.K.S. Wong, Columbia University.
ION CHANNELS: CALCIUM CHANNELS IV

363.7
Modulation of calcium currents by Neuropeptide Y (NPY) in rat retinal ganglion cells (RGCs), Andreas Karschin and Stuart A. Lipton (SPON: H. Wässle, Max-Planck Inst. for Hirnforschung, D-6000 Frankfurt, FRG, and Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115).

Calcium currents were recorded with patch electrodes in both the whole-cell and single-channel (cell attached) configuration. No current was achieved by NPY, as described by Lipton & Tauck [J. Physiol. 1987;385:361].

During whole-cell recording, application of voltage protocols [Fox, N. P. and J. Physiol. 1984;40] suggested the presence of multiple components of calcium current. Test potentials to 40 mV from a holding potential of -80 mV were activated by a slowly inactivating current (t = 10-30 ms). Stronger depolarizations (to >20 mV) activated a more slowly decaying component (t = 100-200 ms) as well as a substantial steady-state level; these components persisted when the extracellular solution was changed from 10 mM Ca2+ to Ba2+.

Some cells displayed only one or two of these components (e.g., transient and sustained) while others had all three. Dihydropyridines yielded the expected results on the sustained (L-type) component. However, unlike the experience in DRG neurons [McClaskey et al., PNAS 1987;84:8359], 10 mM Ca2+ reversibly suppressed all components of calcium current.

363.9
TRANSIENT INHIBITION OF CURRENT BY ω-CONOTOXIN (ω-CgTX VIA) IN RAT RETINAL GANGLION CELLS. Andreas Karschin* and Stuart A. Lipton (SPON: H. Wässle, Max-Planck Inst. for Hirnforschung, D-6000 Frankfurt, FRG, and Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115).

Calcium currents were recorded with patch electrodes in both the whole-cell and single-channel (cell attached) configuration of current was achieved by NPY, as described by Lipton & Tauck [J. Physiol. 1987;385:361].

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363.11

The specific binding of the antagonist NP (2x10^-9 M) to both SPM and PSD fractions was inhibited in a linear fashion by nifedipine (NP) antagonist, by BAY KB644 agonist, and by Sandoz lts. enantiomer, (S)-202-791 agonist and (-)-202-791 antagonist, reaching in all cases 100% inhibition at 10^-8 M. Each of the (+) and (-) enantiomers caused a threefold increase in the NP binding. At concentrations below 10^-9 M, all four components inhibited the binding by >50%. However, when 8x10^-10 M NP was added together, the resulting inhibition was additive, reaching >100%, with both fractions. The same result obtained when the (+) and (-) enantiomers each at 8x10^-10 M were added together. The level of the NP receptor was assessed using the lipid bilayer technique. The electrical activity of the NP receptor was measured at >50-70 mV and opened with increased frequency as the level of the NP receptor was increased. Single-channel blockers were added to the bilayers (side of potential application and vesicle addition). The single-channel blockers were added to the bilayers (side of potential application and vesicle addition) at a concentration of 10^-5 M, and the resulting inhibition was additive, reaching >100%, with both fractions. The same result obtained when the (+) and (-) enantiomers each at 8x10^-10 M were added together. The level of the NP receptor was assessed using the lipid bilayer technique. The electrical activity of the NP receptor was measured at >50-70 mV and opened with increased frequency as the level of the NP receptor was increased. Single-channel blockers were added to the bilayers (side of potential application and vesicle addition). The single-channel blockers were added to the bilayers (side of potential application and vesicle addition) at a concentration of 10^-5 M, and the resulting inhibition was additive, reaching >100%, with both fractions. The same result obtained when the (+) and (-) enantiomers each at 8x10^-10 M were added together. The level of the NP receptor was assessed using the lipid bilayer technique. The electrical activity of the NP receptor was measured at >50-70 mV and opened with increased frequency as the level of the NP receptor was increased. Single-channel blockers were added to the bilayers (side of potential application and vesicle addition).

363.8

Our previous studies showed that Neuropeptide Y (NPY), acting via a GTP-binding protein, inhibited primarily the inactivating component of whole-cell calcium current. Calcium currents were recorded with patch electrodes in calcium influx measured by Fura-2. These preliminary data suggest that NPY acts via a GTP-binding protein in the calcium current.
364.1

EMBRYONIC CHICK MOTONEURONES RESPOND TO NGF IN VITRO AND RETROGRADELY TRANSPORT NGF IN VIVO. D.B. Wayne† and M.B. Heaton. Dept. of Neuroscience, University of Florida, Gainesville, FL, 32610.

The role of NGF in stimulating outgrowth from neurons of the PNS has been recognized. Here we present evidence that embryonic chick motoneurones of the brainstem and spinal cord are NGF-responsive. Brainstem trinalgal and spinal cord lumbosacral neurones specifically transport 125I-NGF transiently in early development and respond to NGF in vitro during that time.

Avidly motoneurones were found to specifically transport 125I-NGF following target (jaw) injections at 4 1/2 and 5 but not at 10 days of incubation. Neurones of the spinal lateral motor column (LMC) were found to specifically transport to 5I-NGF following target (limb) injections at 5 and 6 but not at 13 days of incubation. Dissociates of the trigeminal basal plate (VBP) (day 4) and the ventral spinal cord (SCPB) (day 5) were cultured in the presence of NGF or in control medium. NGF did not affect survival of these populations, however, process outgrowth was enhanced. NGF significantly increased the neurite quantity of SCPB dissociates at 24 and 48 hours while neurite initiation was significantly increased in VBP dissociates after 48 hours. Supported by NIH grant NS-20387.

364.3

SUBSTANCE P MODULATES RELEASE OF LOCALLY SYNTHESIZED NERVE GROWTH FACTOR FROM RAT SAPHENOUS NERVE NEUROMA. U. Otten, F. Keller*, M. Hardung* and D.K. Meyer*. Dept. of Pharmacology, Biocenter of the University, CH-4056 Basel, Switzerland and Dept. of Pharmacology, University of Freiburg, D-7800 Freiburg FRG.

The most proximal segment of the transected saphenous nerve, a neuroma-like structure, was used as a model to study mechanisms in nerve growth factor (NGF) synthesis and release. In saphenous nerve neuronomas of adult rats a long-term increase in NGF protein was detected by an enzyme-linked immunosassay after nerve transection. There was a rapid 9 fold increase in NGF levels 12 h after injury, which reached peak values (9 fold) after 4 days and subsequently fell to 2 fold elevated levels within 3 weeks. Quantitative Northern blots showed, that NGF mRNA levels increased rapidly in nerve tissues, reaching a maximum about 24 days after transection indicating that the increase in NGF in response to injury is due to local biosynthesis.

Superfusion of the neuroma in situ revealed a continuous basal release of NGF protein (Zpg/min) for at least 45 minutes, which was drastically decreased by substance P (SP) in a dose-dependent manner. Maximum inhibition (85 ± 10%, N=8) occurred at a concentration of 0.1 μM. Neurokinin A was less potent than SP. Neurokinin B, as well as other peptides such as calcitonin gene-related peptide, somatostatin and neuropeptide Y at concentrations up to 50 μM did not significantly affect NGF release.

These results suggest that SP may specifically modulate the availability of NGF in the microenvironment of regenerating nerve fiber endings.

364.5


Previous studies indicate that nerve growth factor (NGF) specifically elevates choline acetyltransferase (CAT) in BF cultures. To identify mediating mechanisms, we employed simultaneous specifically elevates choline acetyltransferase (CAT) in BF cultures. To identify mediating mechanisms, we employed simultaneous

364.6


The expression of nerve growth factor (NGF) receptor was studied in rat pituitary gland by immunohistochemistry using 125I-IgG, a specific anti-NGF receptor monoclonal antibody. The NGF receptor immunoreactivity (NGFR) in pituitary gland started to appear diffusely at about 40 days of age. At 2, 4- and 12-month-old, the staining was intense but became diffuse and less distinct in 17-month-old animals. The staining in pituitary was only seen in the posterior but not in the internal and anterior lobes. The staining formed patches in the crest region and in sagittal section of the posterior lobe. The staining was also seen in pituitary stalk and the outer layer of median eminence but was not found in hypothalamic areas. A similar staining pattern was seen in both sexes of 2-month-old rats and castration of males at 30 days did not affect the staining in the adults. 125I-NGF crosslink/125I-IgG immunoprecipitation followed by SDS-PAGE autoradiography, showed that the NGF receptor in pituitary gland had the same molecular weight (90 KD) as previously reported. The amount was much greater in the posterior lobe than in the anterior lobe. Preliminary double labeling results showed that the NGFRII elements in the posterior pituitary were neurinemphi negative. The cellular characterization of NGFR in situ is under further study by both immunofluorescent double staining and by immunoelectronmicroscopy.
634.7 RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR (NGF) FROM HIPPOCAMPUS TO SEPTUM IS IMPAIRED IN AGED RATS. Sooyong Koh and Rebekah Loy. Dept of Neurobiology & Anatomy, Univ. of Rochester, Rochester, NY 14642.

Previous work in our laboratory has demonstrated age-related loss of NGF sensitive neurons in rat basal forebrain (Brain Research 440). Decrease in the number of NGF receptor immunoreactive neurons appears to be a protracted process of chronic cellular dysfunction and not primarily cell death. The present study was undertaken to determine whether this reduction in NGF receptor signifies an impairment of receptor-mediated transport of NGF from target area to the cell bodies of NGF responsive neurons. Female Fisher 344 rats at 29 months of age (n=5) and 13 months of age (n=4) were injected intrahippocampally with horseradish peroxidase and perfused 24 hours prior to aldehyde perfusion and tissue processing for electron microscopy. NGF from target area to the cell bodies of NGF responsive neurons was demonstrated in the cerebral cortex of young adults (n=4) and old (n=3). Labeled neurons in the medial septal nucleus (MS) and ventral medial thalamic nucleus (VDB) were visualized autoradiographically. 15 coronal sections were selected from each brain and nucleus ipsilateral to the injection sites counted. Within the MS/VDB of the aged group, the mean number of radio labeled neurons is reduced 30% compared to young adults (255±48 vs 369±39; p<0.005). This loss of labeled neurons parallels reduction in NGFR in aged rats previously documented. Loss of capacity for retrograde neuronal transport of NGF from their terminals in the hippocampus would result in decrease in NGF delivered to the cell bodies and may promote cellular dysfunction and death of sensory neurons. Supported by NNSA MH-09541 (SK) and ADRA PRG-86-041(RL). Fisher 344 rats were provided by NIA for predoctoral studies (SK).

634.9 EFFECTS OF NGF, CNTF, EGF, PDGF, TGF-β, INSULIN, AND RECOMBINANT HUMAN GROWTH HORMONE ON CHOLINERGIC NEURONS IN CULTURE. B. Kriisel* and H. Neff* (SPON: W. Sharenata), Dept. of Neurology, Univ. of Miami, Miami, FL 33101.

In order to study the trophic control of different populations of central cholinergic neurons, various known trophic factors were added to primary cultures of dissociated septal and pedunculopontine neurons of fetal rat brain. NGF, as shown previously, increased the activity of choline acetyltransferase (ChAT) in septal cultures several fold compared to controls. In contrast, ChAT activity of pedunculopontine cultures, which contain a second major population of centrally ascending cholinergic neurons, was not affected by NGF. However, the clone contained an Arg-Leu substitution at residue -1 that might prevent zymogen activation in a kallikrein and are important for further studies of the α and β subunits of NGF. To investigate specific signals which regulate expression of this trophic factor we have examined the isolated cDNAs provide information on conserved and variable regions of the Mastomys kalilreins and are important for further studies of the α and β subunits of NGF.

634.10 Interleukin-1 Regulates Nerve Growth Factor in Rat Hippocampal Cultures. W.L. Friedlander*, D.L. Larkfort*, T.P. Lira*, and H. Perssen*, Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden, and Dept. of Developmental Biology, Uppsala University, Uppsala, Sweden.

Abundant evidence indicates that nerve growth factor (NGF) is synthesized in rat hippocampus and cortex, and provides trophic support for basal forebrain cholinergic neurons. To investigate specific signals which regulate expression of this trophic factor we have examined dissociated cultures of rat hippocampus from embryonic day 21. Previous work has demonstrated increases in NGF mRNA and protein following lesions of the septo-hippocampal pathway. To determine possible underlying mechanisms, the effect of interleukin-1 on NGF in hippocampal cultures was examined. NGF mRNA was analyzed using Northern blots, and protein was detected using a highly sensitive enzyme immunoassay. Dissociates were grown at a density of one million cells per 35 mm plate for four days, at which time they were exposed to human recombinant interleukin-1 (IL-1) (10 U/ml) for four hours. This treatment elicited a significant increase in NGF mRNA. Since IL-1 has been detected in the brain following injury, it may play a role in mediating the lesion-induced increase in NGF. Studies are now in progress to further characterize this effect.


Nerve growth factor receptor (NGFR) immunoreactivity (IR), as revealed by the monoclonal antibody 192-IgG (Chandler, C. et al. J. Biol. Chem. 259:2885, 1984), is present in the cerebellum of postnatal rat (Eckenstein, F., Brain Res. 446:149, 1988). Its apparent immunocytochemical disappearance from adult cerebellum, however, does not correspond with relatively high levels of receptor IR detected here by radioimmunoassay (Tanishu, M. et al. J. Neurochem. 53:1930, 1988). We have shown that pretreatment of adult rats with colchicine allows visualization of NGFR-IR in Purkinje cells (Plora and Cuello, Brain Res. in press). Here we report the ultrastructural appearance of such IR in these cells of Wistar rats which had received colchicine 24 hours previously at 40 hours prior to aldehyde perfusion and tissue processing for electron microscopic (EM) analysis. The majority of peroxidase IR was localized to the nucleus, although NGF receptor product was also related to Golgi apparatus, rough endoplasmic reticulum and secondary lysosomes. Rarely, immunoreactive cisternae coated vesicles (50-200 nm dia.) were seen near the cell membrane. This is the first EM demonstration of NGFR-IR in neurons of the central nervous system with evidence of NGF synthesis, membrane internalization, and degradation. Supported by the Medical Research Council (Canada).

634.12 NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY PRESENT IN AXIONS AND EPITHELIAL CELLS OF THE RAT SKIN. A. Ribeiro-da-Silva*, R.L. Kenigsberg* and A. Claudio Cuello, Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1V6.

The distribution of nerve growth factor receptor (NGFR) was investigated immunochemically in the skin of the lower lip of the rat. Tissue was fixed by vascular perfusion and then processed for electron microscopy. NGFR immunoreactive sites were revealed with the use of IgG 192 monoclonal antibody followed by a monoclonal anti-mouse IgG/anti-rabbit peroxidase bi-specific secondary antibody. NGFR immunoreactivity was found to occur in a patchy pattern in the plasma membranes of groups of basal keratinocytes of the epidermis and outer root sheaths of hair follicles. Intense immunostaining was also present in the perineural cells and in axons of small cutaneous nerves. Some of these nerves were in close contact with the immunoreactive epithelial cells. Isolated immunoreactive nerve fibers could also be seen penetrating small cutaneous nerves were surrounding the wall of blood vessels and glands. The physiological significance of these findings will be discussed.
TROPHIC AGENTS V


Several recent observations have indirectly led to the suggestion that NGF may have a physiological role for cholinergic neurons of the basal forebrain. We report that rabbit anti-NGF IgG decreases choline acetyltransferase (Chat) activity in the septum and hippocampus of newborn rats. In particular, the anti-NGF antibody, intracerebroventriculally injected (10 µg in 10 µl of phosphate buffered saline) on postnatal days (P1, P2, P4, P6, and P8), produced on P9 a significant decrease (approx. 30%) of Chat activity. This decrease was less evident at P15 (15% decrease), while no significant changes were detected at P5. Furthermore, a marked reduction of Chat-positive neurons was observed in the septal area of 9-day-old rats treated with anti-NGF IgG. At all times, acetylcholine esterase activity remained unmodified. These results provide a direct demonstration that endogenous NGF affects the function of forebrain cholinergic neurons. Thus, a lack of endogenous NGF or a reduced sensitivity to NGF may well underlie alterations and/or loss of these neurons known to occur in some neurodegenerative diseases.

436.13

POSTFUNCTIONAL MONITORING OF ACETYLCHOLINE RELEASE. R.J. Storella, M. Alexander* and S.A. Bloom. Department of Anesthesiology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27109.

Postfunctional monitoring is often used to assess changes in acetylcholine (ACh) release. Thus, the frequency-dependent decrease of either tension or endplate potentials during neuromuscular block suggests a decrease in ACh release. However, we hypothesized that changes in ACh release in the presence of α-BTX (αBT) may be masked by other events due to αBT’s irreversible binding to ACh receptors. The mouse phrenic-nerve diaphragm was maintained in a modified Krebs solution, aerated with O2/CO2 (95%/5%) at 37°C. The nerve was stimulated supramaximally and isometric tension recorded. Addition of atropine (0.2 Hz) block. In different cells, ACh elicited either depolarizations or hyperpolarizations. These results suggest that ACh acts at muscarinic receptors on basal forebrain cells to produce two types of response. Supported by NIH EY0302 and ADRDA H-86-099.
HALF-LIFE OF PHOSPHATIDYLCHOLINE IN CHOLINERGIC AND NON-CHOLINERGIC NEURONS. W.D. Blaker and A.F. Dobrenski* VA-MD Regional College of Veterinary Medicine, VA Tech, Blacksburg, VA 24061.

In order to determine whether peptidergic afferents to forebrain cholinergic neurons (ChAT) compete with the cholinergic synapse, rats were processed for the simultaneous light microscopic visualization of peptidergic fibers/terminals and chol. acetylcholinesterase (ChAc) containing neurons, using nickel enhanced DAB for peptidergic terminals and DAB for identification of ChAT containing neurons. Antibodies against cholinergic (Colchicine) and peptidergic markers were developed. There is a differential distribution of the various peptidergic fibers to the subdivisions of the cholinergic forebrain system. For example, peptidergic neurons in the ventral pallidum are situated in a network, and ChAT neurons in the bed nucleus of the stria terminals may receive ALF-H1 fibers. These neurons are supplied only occasionally by other peptidergic afferents.

On the other hand, peptidergic neurons in the substantia innominata receive a heavy input from virtually all of the peptidergic systems. Under voltage clamp recording studies confirmed the presence of NPY-containing terminals on ChAT cell bodies and light microscopic evidence suggests that these terminals project to the NPY-containing neurons. Supported by USPHS Grant NS 23945 and 17743.
365.11 IMPACT OF PLASMA ANTICHOLINERGIC ACTIVITY ON COGNITIVE PERFORMANCE OF ELDERLY AMERICAN PATIENTS. M. Unger, A. Lipton, F. P. Zemlan. Lab. of Geriatrics, Univ. of Cincinnati, College of Medicine, Cincinnati, OH 45267-0559.

An exploratory pilot study on the relationship between anticholinergic plasma activity and cognitive measures in geropsychiatric inpatients (mean age 59 years). Subjects received various psychotropic medications with known antimuscarinic properties. Plasma samples were obtained when patients were medication-free, and five to seven days after target dosage had been achieved. Samples were analyzed for total anticholinergic activity, using the tritiated quinuclidinyl benzilate ([3H]QNB) radio ligand binding assay (Lune, L., and Coyle, J. T., Psychopharmacology, 75: 1-9, 1981). Results were expressed in units of atropine equivalence [pmol/ml].

Non-demented subjects (n=10) developed no cognitive dysfunction while their mean anticholinergic plasma activity reached 2314 pmol/ml (5.0±125). In demented patients, meeting diagnostic criteria for probable Alzheimer's disease (McKhann, G., et al., Neurology, 34: 485, 1984), aggregate cognitive rating scores revealed that cognitive performance ratings deteriorated an average of 18 percent while anticholinergic plasma activity increased to 2700 pmol/ml (5.0±1957).

365.13 CHOLINERGIC NEURONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN THE ADULT RAT: A TOPOGRAPHICAL STUDY. M. Plotkin, P. Kakari, and A. C. Guillo. Dept. of Pharmacology and Therapeutics, McGill University, Montreal (Quebec), Canada H3G 1Y6

We attempted to define the morphological landmarks of the rat nucleus basalis magnocellularis (NBM) based on the topographical organization and morphology of its cholinergic perikarya. The brains of 4 male Wistar rats were perfused and sequential 50 μm thick coronal sections of the entire NBM were cut, processed for choline acetyltransferase (ChAT) immunocytochemistry, and studied with an image analysis system (Quantimet 920). Neuronal density as well as cell size and shape were assessed. Results indicated that in both the rostral and caudal thirds of the NBM the cholinergic neurons are distributed along a band following the ventro-medial edge of the globus pallidus. In the mid third of the nucleus cell distribution assumed a triangular outline and cell density was lower than in the other two thirds. However, cholinergic perikarya were larger in the mid NBM than in the rostral or caudal third where they were of equivalent size. Cell roundness measurements suggested that the shape of the ChAT immunoreactive nerve cell bodies remained constant throughout the entire rostro-caudal extent of the NBM. Thus the NBM can be readily identified within the rat basol forebrain based on the topographical distribution and density of its cholinergic neurons. (Supported by MRC and FRSQ).


The degree of depletion of cortical choline acetyltransferase (ChAT) in rats with excitotoxins lesions of the nucleus basalis magnocellularis (NBM) has never been correlated with the extent of their learning and/or memory deficit. In Alzheimer's disease, the degree of dementia has been correlated with the loss of cortical ChAT. We report that acquisition and accuracy of performance in a water maze task is highly correlated with the reduction in cortical ChAT. T-544 rats (n=12) were anesthetized and bilaterally lesioned in the NBM (2 sites/side) with kainate in 2 stages 1 week apart. Three weeks post-surgery the 5 surviving lesioned and 8 sham control rats were tested in a water maze (10 trials/day, 2 trials/day for 5 consecutive days, followed by 2 days off, and then tested for 5 more days. The following week, rats were tested for 72 h retention (600s max.) of a passive-avoidance (PAL) task (6 trials/day). Two days after initial maze testing, the rats were tested for maze retention (1 trial block). The next day, after 1 trial block, the platform was removed and the rats swam for 90s for a spatial probe trial. The NBM lesioned rats had a significant reduction in cortical ChAT activity (mean ±24% depletion). The NBM lesioned rats displayed a severe deficit in acquisition of the water maze task. Neither group displayed a reliable difference in performance the following week, rats were tested for 72 h retention (600s max.) of a passive-avoidance (PA) task (6 trials/day). These results suggest that cortical ChAT depletion in NBM rats is associated with a severe deficit in acquisition of the water maze task. We have also demonstrated that a severe learning and memory deficit occurs in rats with NBM lesions (n=7). This is the first report of a significant correlation between a learning paradigm and degree of cortical ChAT depletion in the rat. These data suggest that the observed ChAT depletion is important for the behavioral deficit.


Garden Warblers show distinct annual cycles of testicular development and regression. Our goal was to determine if the components of the hypothalamic-pituitary-gonadal system change temporally through phases of the annual cycle. We found distinct autonomous seasonal variation in hypothalamic GnRH-I content in warblers who kept on constant daylight. GnRH-I content is low from December through March, but increases in April and again in June to May. Of particular interest is that the finding that GnRH-I content does vary in individuals during the 7 months preceding breeding, even in the absence of photoperiodic change. Although pituitary LH content increased from March to April, levels varied only 2.5 fold during the study. Seasonal changes in plasma LH levels paralleled the changes in hypothalamic GnRH-I content; levels of both increased steadily from March to June. Increases in testicular mass occurred in April and again from May to June. Increases in the androgen testosterone production paralleled increases in mass. Thus, in warblers a series of autonomous hormonal changes in season, regulating seasonal gonadal growth. Supported by the Alexander von Humboldt Foundation.

366.2 EFFECTS OF CHRONIC CLONIDINE ADMINISTRATION AND WITHDRAWAL ON CIRCADIAN ACIVITY RHYTHMS IN RATS. A. M. Rosenwasser. Dept. of Psychology, Univ. of Maine, Orono, ME 04469.

The alpha-2-adrenergic agonist clonidine has been shown to alter mood, arousal, and activity. While acute administration is generally sedating, chronic administration can result in hyperactivity and irritability. Furthermore, recent studies have found that clonidine withdrawal results in a long-term behavioral depression. In light of the reported relationship between mood and activity disorders and circadian disruptions, these results suggest that chronic clonidine administration and withdrawal may affect free-running circadian activity rhythms. After three weeks of clonidine treatment (30 mg/kg/day), animals were exposed to dim light during the subjective day. During clonidine administration, free-running periods were shortened, circadian amplitudes were reduced, and overall activity levels of activity were lower, relative to baseline. When the drug was withdrawn, animals treated with shorter, circadian amplitudes were reduced, and overall activity levels of activity were lower, relative to baseline. When the drug was withdrawn, animals treated with shorter, circadian amplitudes were reduced, and overall activity levels of activity were lower, relative to baseline. When the drug was withdrawn, animals treated with shorter, circadian amplitudes were reduced, and overall activity levels of activity were lower, relative to baseline.
We have recently observed that chronic clorgyline (CLG), a monoamine oxidase inhibitor with antidepressant properties in humans, increases the period of wheel-running and alters the phase response curve to fifteen minute light pulse administration of CLG in drug-free hamsters. These experiments indicate that CLG differentially alters the response of the circadian pacemaker to light. For example, in the period of wheel-running, the induced phase-delay was increased and the magnitude of light-induced phase-advances was decreased by chronic CLG treatment in the earlier study (29). The light intensity selected for the intensity-response relationship between light and the phase-shift response is unknown. In order to examine the relationship between CLG and responsiveness of the circadian pacemaker to light, the phase-shift response to saturating, five minute pulses of light at various times of the day was examined.

Group-housed hamsters were implanted with Alzet minipumps (Model 2002) containing either CLG (20 µg 2.5 mg.kg⁻¹.day⁻¹ for four weeks). Individual hamsters were then transferred to cages and had free access to running-wheels, food and water. After about one week in LD 14:5 (5) hours, hamsters were exposed to continuous darkness (DD) for the next seven days. On day 8 hamsters were transferred to a light chamber which a five minute pulse of monochromatic light (506 nm, to 1000 µW.cm⁻²) was delivered either at CT 13.5 or CT 18. (The phase-shifting effects of the benzodiazepines on circadian rhythmicity.

...selectively greater activity during REM sleep. (Supported by H L 22418-10)


Obstructive sleep apneas in humans may result from relaxation of the tongue toward the posterior wall of the pharynx during REM sleep; since activity of the genioglossus muscle, the principal tongue protruder, greatly diminishes during that state. During waking and quiet sleep, genioglossal EMG activity phasically increases during inspiration in the awake state. During REM sleep, both phasic and tonic activity decrease dramatically. The nucleus reticularis gigantocellularis (NRC) may hyperpolarize XII nerve motoneurones during REM sleep. Stimulation of the NRC causes IRSPs in somatic motor neurones only during REM periods. NSC neurones also demonstrate selective rhythmic activity during REM sleep. We hypothesize that hypoglossal motoneuronal activity will demonstrate selectively greater activity during REM sleep. The nucleus reticularis gigantocellularis (NRC) may mediate state-specific effects on hypoglossal activity. (Supported by HL 22418-10)
366.9
COMPUTER NETWORK RECONSTRUCTION OF PROJECTION POPULATIONS TO CHOLINERGIC NUCLEI IN THE Rhesus Monkey.
J. Quattrrochi, T. Hensch,*, A. Manela*, and J.A. Hobson.
Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.
We define the precise multicellular network projecting to a discrete injection site within the anterodorsal pontine tegmen tum in the rhesus monkey using retrogradeabeled cells or two-photon microscopy. Each projection site is identified by local and systemic immunohistochemistry, in situ hybridiza tion, and computer reconstruction. The network contains diverse projection populations, including connections to brain stem, hypothalamic, thalamic, and cortical regions. The network is circuitry-dependent, with axonal connections that extend throughout the brain. This network is organized in a centrocaudal gradient, with a dense projection from the anterodorsal pontine tegmentum to the midbrain, and a sparse projection to the neocortex. The network is dynamic, with plasticity that occurs in response to changes in brain function. The network is a key regulator of behavioral states and cognitive functions.

366.11
LOW SODIUM DIET ELEVATES PLASMA NOREPINEPHRINE (NE) AND IMPAIRS SLEEP PATTERNS IN MAN: A REPLICATION.
(Sponsor K.F. Moe). Psychiatry and Behavioral Sciences and Medicine, University of Washington and Seattle and American Lake VAMC, Seattle, Washington 98195, USA.
Previously we reported that a low sodium diet significantly elevated plasma NE and reduced sleep efficiency, compared to a normal sodium diet. To replicate these findings, we studied the effect of a low sodium diet on sleep efficiency in a group of healthy young men. We found that the low sodium diet significantly increased plasma NE and decreased sleep efficiency. The results suggest that low sodium diet may affect sleep by increasing plasma NE levels and reducing sleep efficiency.

366.12
CEREBRAL GLUCOSE METABOLIC STUDY OF GENERALIZED ANXIETY DISORDER AS ASSESSED BY PET. M. Vasquez*, J.C. Mc Brien*, C. R. The*, and D. D. Ral&&.*
Dept. of Psychiatry, UCI, Irvine, CA 92717
INTRODUCTION A study of regional cerebral metabolism of anxiety as assessed by positron emission tomography was performed. Twenty patients who met DSM-III-R criteria for generalized anxiety disorder were scanned with a PET scanner. Without panic attacks were compared with nineteen normal controls. Subjects were given 5 mg of 18 FDG and told to fixate on a red light for 1 hour. Subjects were then scanned on a PET scanner. RESULTS There was a significant decrease of relative glucose utilization in the left side of the cerebral cortex in GAD patients versus controls (significant hemisphere by group interaction, F=5.37, p<0.01). The anterior cingulate, basal ganglia structures such as the putamen and left globus pallidus showed a significant decrease. Deep layer structures such as amygdala, the hippocampus, and parahippocampus showed no significant change in GAD patients compared to controls. Thalamus and structures showed no significant change in GAD.

366.13
SPECTRAL ANALYSIS OF PREMENSTRUAL TENSION SYMPTOMS.
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Premenstrual syndrome refers to cyclic changes in mood and physical symptoms in the late luteal phase of the menstrual cycle. Characteristics include irritability, depression, fatigue, and premenstrual tension. A study was conducted to examine the relationship between menstrual cycle phases and severity of premenstrual tension symptoms. Premenstrual tension was assessed using the Premenstrual Assessment Scale (PAS). The PAS is a self-report measure that assesses symptoms across four domains: physical, emotional, cognitive, and behavioral. The PAS is scored on a 0-7 scale, with higher scores indicating greater severity of symptoms. The study found that premenstrual symptoms are significantly worse during the late luteal phase of the menstrual cycle, compared to the follicular phase. The findings provide evidence for the cyclic nature of premenstrual symptoms and suggest that hormone levels play a role in the development of these symptoms.

To complement our morphological studies of identified sensory neuron presynaptic terminals, we have examined the effects of long-term sensitization on the structure of an identified postsynaptic target -- the gill motor neuron L7. Individual motor cells from control (N=2) and sensitized animals (N=4) were labeled with HRP and serially reconstructed. Preliminary I.M. observations suggest an increase in focal outpocketing of finger-like processes (spines) in sensitized cells. Quantitative ultrastructural analysis of 943 labeled profiles revealed an increase in the frequency of presynaptic contacts on L7 processes in sensitized compared to control animals (0.43 ± 0.03 S.E.M. vs. 0.78 ± 0.02, t=3.32, p<0.01). The number of synaptic contacts increased (1.72 ± 0.06 vs. 0.53 ± 0.09, t=10.54, p<0.001) as does the incidence of multiple synaptic contacts onto the same postsynaptic profile (0.06 ± 0.008 vs. 0.016 ± 0.001, t=3.8, p<0.05). Combined, these data indicate a striking upward shift following long-term training in the percentage of L7 surface area that is occupied by synaptic contacts (0.08 ± 0.002 vs. 0.02 ± 0.006, t=11.8, p<0.001).

These results are consistent with our observations of an increase following sensitization in sensory neuron synapses and provide additional support for the notion that changes in synapse number may represent a mechanism underlying long-term memory.


Activity-dependent enhancement of heterosynaptic facilitation at sensorimotor synapses has been proposed to be the cellular mechanism underlying classical conditioning in Aplysia. To study further the molecular mechanism underlying this synaptic plasticity, we have examined the interaction between spike activity in sensory neurons and facilitation produced by application of 5-HT (100 Â¼M).

We measured the amplitude of the excitatory synaptic potential (EPSP) evoked in motor cell L7 and 10 min later treated the cells with either a) control treatments; b) 5-HT puff; c) tetanus to sensory neuron (20 Hz for 2 sec); or d) tetanus plus 5-HT puff 1 sec after onset of tetanus. The EPSP was measured at 1, 5, 10, and 15 min after the respective treatments. Each treatment significantly enhanced the EPSP after 1 tetanus compared to controls (range of +27 to +62% compared to -14%, N=10). At 15 min, the EPSP following treatment with tetanus plus 5-HT (+11%) was enhanced relative to treatment with 5-HT (+9, +3%), tetanus (-26, 3%) or controls (-41 ± 27%). One needs to determine whether this prolongation of synaptic enhancement requires the temporal pairing of the two stimuli, and whether other facilitatory transmitters can substitute for 5-HT.


We have developed an intact-cell protein kinase assay for clusters of pleural sensory neurons based on the analysis of proteins labeled by quantification of 35S-methionine (a protein synthesis inhibitor) or actinomycin D (an RNA synthesis inhibitor) during the 1.5-hr training period. This finding suggests that genes and proteins required for long-term memory formation that are not needed for the short-term process. We therefore studied the effect of 5-HT on total protein synthesis in the pleural/sensory cells by using incorporation of 35S-methionine (150 Â¼M) into the TCA-precipitable fractions. Application of 3 um 5-HT during the training period (1.5 hrs) induced three temporally distinct changes. The first change resulted in a decrease of 30-75% following pulsing of 3-5-HT or cAMP for 2 hrs, which produces electrophysiological effects lasting 24 hrs, caused an increase in phosphorylation of 2 proteins (170 kDa and 160 kDa), and 5-HT or cAMP block application blocked the increase in protein phosphorylation observed at 24 hrs, without affecting the increase in phosphorylation in response to a 2 min 5-HT treatment. These data suggest that in addition to their role in short-term facilitation, 5-HT and cAMP can recruit a long-term mechanism for a persistent increase in protein phosphorylation that is dependent for its induction on active translation and transcription.


We have developed a protocol in which comparable short duration synaptic enhancement was evoked by 5-HT, SCP-B, and tetanic stimulation, as 5-HT, can evoke long-term changes in sensorimotor synapses.

We first developed a protocol in which comparable short duration synaptic enhancement was evoked by 5-HT, SCP-B, and tetanic stimulation. After testing the amplitude of the excitatory synaptic potential (EPSP) evoked in motor cell L7, each culture was given one of the treatments 4 times at 20 minute intervals. Whereas 5-HT significantly enhanced the amplitude of the EPSPs when retested 24 hrs later (55±11%, N=6), treatments with SCP-B (7±10%, N=6) or tetani (6±6%, N=6) did not significantly affect synaptic strength and were similar to the control untreated group (4±6%, N=6).

These results, which parallel those from short- and long-term synaptic depression, suggest that the long-term modulation of this identified synapse may be evoked only by a subset of the neuregulators that can produce short-term synaptic plasticity.


The long-term facilitation (LTF) induced by 5-HT in Aplysia sensory neurons is blocked by the application of a protein synthesis inhibitor (50 Â¼M actinomycin D or 1 Â¼M anisomycin) during incorporation of 35S-methionine (150 Â¼M) into the TCA-precipitable fractions. Application of 3 um 5-HT during the training period (1.5 hrs) induced three temporally distinct changes. The first change resulted in a decrease of 30-75% following pulsing of 3-5-HT or cAMP for 2 hrs, which produces electrophysiological effects lasting 24 hrs, caused an increase in phospho-

Long-term sensitization of the gill and siphon withdrawal reflex in Aplysia, produced by one or four days of training, is accompanied by a specific increase in the incorporation of S-methionine into four proteins (Castellucci et al., Neurobiol., Suppl., 1984). One of these, Aplysia protein #407, MW 52kD, pi 4.7, is present in sensory neurons and can be identified on coomassie blue-stained preparative D-Gels of Aplysia total CNS extract. This protein was isolated from preparative gels and sequenced using a gas phase microsequencer. We obtained 16 residues of amino acid sequence (XPATYKKFEQFLODXAE). A sequence homology search in protein sequence databases revealed no homologous proteins. However, we similarly isolated and sequenced a comigrating protein from a crude cell lysate of cultured rat embryo fibroblasts obtaining 15 residues of amino acid sequence (XPATYKKFEQFLODXAE). The Aplysia and rat proteins are 64% identical at the amino acid level. We have synthesized oligonucleotides which correspond to all possible coding sequences of 7 residues in the Aplysia peptide (VPKFEQFG). We are currently using this oligonucleotide mixture to screen lambda gt10 cDNA libraries prepared from both Aplysia total CNS and Aplysia abdominal ganglion sensory and motor neurons.

SEQUELAR CHANGES OF IONIC CURRENTS DURING CLASSICAL CONDITIOING OF HERMISIDENDA. C. Collin et al. Lab. of Molecular and Cellular Neurobiology, NICHD-NIH, Rockville MD 20852.

Voltage-dependent current (I, I., I., and C.) and a Ca current (I.) in E. S. receptors persist for 48h after classical conditioning (Collin et al., Science, 231: 3542, 1986). Here, we studied changes in these currents which depend upon increasing numbers of trials. After 1 Akt, 1 BPL and 1 CBL were used, a Ca current (I.) was reduced by about 70% compared to controls. At 0.7 ms, a Ca current (I.) was reduced by about 70% compared to controls. Antidromic spikes in the APL were observed with 500 Hz stimulation, but no action potentials were observed with 100 Hz stimulation.


The dependence on Mg2+ of protein kinase C (PKC) binding to membranes is complex: it is blocked by physiological Mg2+ concentrations, but reversed by Ca2+ and phorbol ester. The translation produced by phorbol ester is maximal at 3 mM Mg2+. Changes in intracellular Mg2+ may be important in regulating PKC in Aplysia. In any case, the concentration of Mg2+ must be optimized to obtain consistent data on the role of the kinase in facilitating Aplysia neurons. We examined unilateral sensitization of the tail-withdrawal reflex (mediated by pleural sensory neurons) with the protocol of Scholz & Byrne (Science 235: 685-687, 1987), shocking one side of the animal. Facilitation of sensory cells also is produced by serotonin or phorbol ester. We find that all of these facilitating stimuli activate PKC, translocating the enzyme from cytosol to membrane.

SEQUELAR CHANGES OF IONIC CURRENTS DURING CLASSICAL CONDITIOING OF HERMISIDENDA. C. Collin et al. Lab. of Molecular and Cellular Neurobiology, NICHD-NIH, Rockville MD 20852.

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Adenylate cyclase has been suggested as a molecular site of convergence for Ca2+ and serotonin, the cellular representations of the CS and US in classical conditioning of the gill and siphon withdrawal reflex of Aplysia. By modifying a recently developed, perfused membrane cyclase assay, we were able to study the temporal interactions of brief pulses of Ca2+calmodulin and serotonin in stimulating adenylate cyclase from Aplysia CNS. The apparatus enabled continuous monitoring of cyclase activity and rapid application and termination of ligand stimuli. We found that the magnitude of adenylate cyclase activation by 6 sec pulses of Ca2+ and serotonin depended on their order of presentation: cyclase activation was 25% to 50% greater when the Ca2+ transient immediately preceded the serotonin transient (Forward pairing) than when they were given in the reverse order (Backward pairing). The magnitude of the Forward-Backward difference depended on the concentration of Ca2+ used and on the presence of calmodulin in the assay. When a non-hydrolyzable GTP analog was used, no Forward-Backward difference was observed. These results suggest that adenylate cyclase may provide a molecular mechanism underlying the two requirements for contiguity detection in classical conditioning: temporal proximity and order dependence.

PROTEIN AND mRNA CHANGES INDUCED BY ASSOCIATIVE CONDITIONING OF HERMISIDENDA. T. Nilson and D.L. Akson. Lab. of Molec and Cellular Neurobiology, NICHD-NIH, Bethesda, MD 20892 (SPON: A. Sidhu).

Protein synthesis inhibition enhances Ca2+ -mediated reduction of the K+ currents which undergo persistent reduction with classical conditioning of Hermisenda [Akson et al., PRAS (1987)]. To assess the role of specific proteins in classical conditioning trained with paired light and rotation. 24 h after training, eyes were dissected and incubated for 6 h with mixed 3H amino acids and [32P]phosphate, and proteins were analyzed by HPLC. Conditioning increased a 21 kDa phosphoprotein (previously found to be a C-kinase and CaM-kinase substrate) by 1.6-fold compared to control, with random and naive controls. The tritiated protein was increased 5-fold compared to control, with random and naive controls. The phos. pool of the 21 kDa protein was increased 0.7 to 0.9 normal levels (p<0.001). The 21 kDa protein was incorporated into the 169 kDa protein was increased 5-fold (p<0.001) while the sp. act of the 21 kDa protein was decreased to 0.7 to 0.9 normal levels (p<0.001). No differences were observed between random and naive controls. The above changes were significantly correlated with the degree of learning (p<0.01). In a related experiment, eyes from trained H were incubated with 32P-phosphate and mRNA was isolated and analyzed on agarose gels. The mRNA showed changes which paralleled the protein changes for the 21 and 169 kDa proteins. Thus, associative learning in H changes the synthesis rates of specific phosphoproteins within H eyes. These results are consistent with previous results demonstrating a correlation of mRNA levels with learned behavior in intact H up to 4 days after conditioning.
MECHANISMS IN CENTRAL POST-STROKE PAIN (CPSP) - A CLINICAL STUDY.


Central pain can be induced by thalamic as well as extrathalamic cerebrovascular lesions (CVL). To elucidate the mechanisms of CPSP the present study investigated to what extent the CVL engages the thalamus in patients with CPSP, which other locations of CVL can cause CPSP, if the pain differs according to the location of the CVL and which neurological symptoms and signs the patients (pts) have in addition to pain.

Patients and Methods. Examinations including quantitative sensory tests were done of 20 men and 7 women.

Results. The lesions involved the thalamus in 33 % of the pts. In 27 % and 22 %, respectively, the CVL were located infratentorially and supratentorially. The pain was usually burning, aching, prickling or lacerating with some differences due to CVL location. It was increased by various stimuli. Abnormal temperature sensibility was the only sign common to all pts. 93 % had some hypertensitivity to noxious heat. Hypersensitivities to cutaneous stimuli: hypesthesia to touch and vibration was found in 52 % and 41 %, resp. 48 % had pareses.

Conclusion. The results indicate that the crucial factor for CPSP is a lesion affecting temperature (and possibly pain) sensibility. It appears likely that the spinothalamic tract or its relay or thalamocortical projections for pain differ according to the location of the CVL and which neurological symptoms and signs the patients (pts) have in addition to pain.

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368.5

**TWO COMPONENTS OF PAIN SENSATION IN RESPONSE TO COLD STIMULATION OF HUMAN TEETH**

K. D. Kniffki, E. Jyväsjärvi*, M.K.C. Menapé* and A. Sturhanhoff*

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In animals it has recently been demonstrated, that cold stimulation of teeth elicits quite distinct responses in intradental Aδ- and C-fibres (Jyväsjärvi, E. and Kniffki, K.-D., J Physiol. 391: 195-207, 1987). The aim of the present study was to evaluate, whether these different response properties of the intradental Aδ- and C-fibres are reflected in distinguishable pain sensations in humans.

Randomly adjusted cold stimuli between +25°C and -40°C lasting 2.5 min were applied to 13 upper central incisors. The subjects had to rate the magnitude of their pain sensations using a verbally anchored 50-point scale (category partition), and afterwards described their sensations in 56 sentences.

Almost all subjects rated two components of pain. The magnitude of both pain components was dependent on the temperature of the stimulating individual axons and their receptive field characteristics. The receptive field characteristics of 19 of the Aδ and C fibers were identified by electrical current pulses applied to the periodontal space, the dental pulp, and in some experiments also to the nerve trunk.

Fifteen of 260 identified slowly conducting periodontal fibres were also activated by cold stimulation. These fibres were classified as C- and four as Aδ-fibres. The response properties of the fibres to non-electrical stimulation of the periodontium were similar to those of slowly conducting afferent fibres (Kniffki, K.-D., personal communication). The responses to occlusal pain elicited by electrical current pulses applied to the periodonatal space included a C-fibre component (cf. Bennett, G. and Bennett, N. J., NAB, NIDR, NIH, Bethesda MD 20892. U.S.A.).

The receptive fields of the single C-fibres, determined with a dental probe, were located deep in pulp and periodontal tissues. In a few cases, after determining the branch of the fibres' responses, the pulp was completely removed and the responses to stimulation of the periodontal ligament were found to persist.

It is concluded, that there exist some slowly conducting fibres in the inferior alveolar nerve, that branch to innervate more than one oral tissue as it has been shown in other peripheral nerves.

368.8

**THE DEACTIVATION OF C-FIBER NOCEPCIIVE RECEPTORS FOLLOWING INTRADERMAL INJECTION OF THE CAPSAICIN ANALOGUE NE-21610.**


The potency, specificity, and time course for excitation and deactivation of capsaicin receptors following i.d. injection of the capsaiacin analogue NE-21610 was determined in neurophysiological experiments in anesthetized monkey. Single fiber recordings techniques were used to monitor the heat and mechanical responsiveness of primary afferent neurons before and after 30 µl injections into the receptive field (RF). Four C-fiber nociceptors received a 0.3 µg dose which resulted in: (1) a brief (10 s) discharge, (2) a period (5-120 min) of complete insensitivity, and (3) a subsequent recovery. Injection of 3 µg into the RF was similarly brief, however the deactivation lasted more than 240 min. In comparison, a 3 µg injection of capsaicin (n=5) produced a deactivation that lasts from 5 to 30 min. Injections adjacent to the RF of C-fiber nociceptors resulted in a small response (10-20 discharges), but no deactivation. In addition, other C-fiber afferents whose RF could not be located with mechanical stimuli were activated by injections. These afferents could be chemospecific. 3 µg dose of NE-21610 did not activate or alter the responsiveness of Aδ-fiber nociceptors (n=4) or low-threshold mechanoreceptors (n=2). These results suggest that, in comparison to capsaicin, the selective deactivation of C-fibers by NE-21610 is at least 10 times more potent and long lasting.

368.10

**ALTERED PREPROTACHYKININ GENE EXPRESSION IN TRIGEMINAL AND CERVICAL DORSAL ROOT GANGLIA IN RESPONSE TO SUBARACHNOID HEMORRHAGE.**

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Substance P (SP)-containing projections from trigeminal and dorsal root (DRG) ganglia innervate cerebral vessels and may be involved in the transmission of pain sensations associated with cerebrovascular events. SP in the subarachnoid space. We monitored neuropeptidp levels in the cerebral arteries, trigeminal ganglia and DRG, as well as preprotachykinin mRNA levels in trigeminal ganglia, following the intracistemal injection of blood. Marked, 50% decreases in basilar artery SP levels were found within 4 h. SP in the DRG was depleted at 48 h and persisted for 7 days. The middle cerebral artery and circle of Willis were exposed to less blood and normal SP levels were maintained. In trigeminal ganglia, levels of SP were increased at 24 and 72 h. The preprotachykinin mRNA levels, as assessed by northern analyses, were also elevated in the trigeminal ganglia at 48 h. To investigate the pathophysiological mechanism, we dispersed cultured F-11 cells (neuroblastoma x dorsal root ganglion) were exposed to hemorrhagic (1h) and hypoxic (1 h) conditions. The expression of preprotachykinin mRNA levels in the cerebral veins and SP in the subarachnoid space were upregulated in the periodontal space of the capsule in cells. These results are consistent with blood-induced alteration of preprotachykinin gene expression in cerebrovascular sensory fibers, and implicate these fibers in the pathophysiology of subarachnoid hemorrhage. Supported by NS00166, NS10828, The McKnight, Sloan and American Parkinson's Disease Assts.
PAIN PATHWAYS

368.11 RECEPTIVE FIELD PLASTICITY OF DORSAL HORN CELLS: A COMPARISON BETWEEN MULTIPLEXED AND NONPLEXED HIPPOCAMPAL NEURONS. J.M. Laird and F. Gervero (SPIN: European Neuroscience Association) Department of Physiology, University of Bristol, Medical School, Bristol, U.K.

It is known that the receptive field (RF) size of some dorsal horn cells increases following a brief period of noxious stimulation. We have now investigated such RF changes in parallel in two populations of neurons, multiplexed (Class 3) and nonplexed (Class 3) neurons in the sacral dorsal horn of the rat. The conditioning stimuli were 0.5 N pinches delivered to the neuron's RF, the tail over a period of up to 5 minutes and/or electrical stimulation of A and C fibres from the tail. RFs were measured and mechanical thresholds determined before, during and after these stimuli. Following a single noxious pinch the RFs of all Class 3 neurons decreased and mechanical thresholds were decreased. Similar changes were seen after electrical stimulation, as has been previously described.

In contrast, Class 3 neurons exhibited much smaller increases in RF size which were restricted to the immediate area around the noxious pinch. Mechanical thresholds fell and excitability increased in some neurons but to a lesser degree than in the Class 2 cells. In about 50% of the cells several pinches were needed before changes were evident.

We conclude that RF changes in Class 3 cells following noxious stimulation can be explained by sensitization of primary afferents whereas those observed in Class 2 neurons include an important central component. This shows fundamental differences in the processing of nociceptive information by multiplexed and nonplexed neurons.

Supported by grants DIUC 84/87 and FONDECYT 698.

POSTSYNAPTIC MECHANISMS III

369.1 INTERACTIONS AND POSSIBLE SECOND MESSENGER SYSTEMS INVOLVED IN NEUROTRANSMITTER RESPONSES IN THE THALAMUS. David A. McCormick, Yale University School of Medicine.

ACh and NE are known to both decrease a resting gK, while ACh and the GABA agonist, baclofen, can both activate a gK in thalamic neurons. The possibility that these actions are achieved through the same ionic channels and/or second messenger systems was investigated using intracellular recordings in relay neurons of the guinea pig lateral geniculate nucleus. In vitro, sequential application of ACh, NE and baclofen revealed that the ACh and NE-induced inward currents were fully reversed at 100 µM as the ACh and baclofen-induced outward currents, displayed non-additivity indicating that these three transmitters converge upon only two gKs. Application of the muscarinic antagonist scopolamine blocked the ACh responses, but not those of NE or baclofen. L-glutamate responses to picrotoxin (50 µM) displayed (in vitro) only the inward currents in response to ACh and NE and no response to baclofen. Similarly, intracellular injection of GTP-γS (200 µM) blocked the outward currents induced by ACh and baclofen, but not the inward currents induced by ACh and NE. Intracellular injection of the calcium chelating agent BAPTA blocked the medium AHP, but not the ACh and NE responses. These results indicate that ACh and GABA activate the same gK in thalamic neurons through a pertussis toxin sensitive G protein, while NE and ACh decrease the same gK through an as of yet unknown second messenger system.

369.2 ACTIVATION OF PROTEIN KINASE C REDUCES GABA-A MEDIATED CHLORIDE CONDUCTANCE. A.Steiger* and R.R.R. Wong, Department of Neurology, Columbia University, New York, N.Y. 10032.

There is increasing evidence that ligand-gated receptor-channel complexes are regulated by protein phosphorylation. We have examined the relationship between phosphorylation factors (Neurosci. Abstr. 13, 279.7) and phosphokinase C activation by GABA-A receptor function in the regulation of GABA-A receptor function. We examined the action of phorbol 12-13 dibutyrate (PB) as a stimulus to activate GABA-A receptors in guinea pig thalamic neurons and in isolated cells prepared from the CAT region of the guinea-pig hippocampus (Kay and Wong, 1986). Whole-cell voltage clamp experiments were performed at room temperature, cells were clamped at -100 mV. GABA (200 µM) was applied by short pressure pulses (20-80 ms). Application of phorbol 12-13 dibutyrate (250 nM) via perfusion reduced the peak current amplitude of the GABA-mediated outward currents to 69 ± 5.7% (N=5) and reduced GABA currents recovered partially (81.5% ± 5.2, N=5, compared to 63.8 % ± 4.7 during phorbol 12-13 dibutyrate application in these 5 cells). These data indicate that activation of protein kinase C by phorbol 12-13 dibutyrate reduces GABA-A mediated chloride conductance. Taken together with previous findings (Neurosci. Abstr. 13, 279.7) it may be speculated that the GABA-A receptor is regulated by several protein kinases, as reported for the nicotinic ACh receptor (comp. Huganir and Greenberg, TIPS, 1997).

Supported by grants from NIH and Klingenstein Foundation.

369.3 MODULATION OF ELECTROTONEIC COUPLING BETWEEN HIPPOCAMPAL NEURONS BY NMDA AND C-KINASE. M. O'bearn and B.A. Calgren, Department of Physiology, University of Calgary, Calgary, Alberta. T2N 4N1

Electrotoneic coupling and dye coupling has been observed between hippocampal neurons in brain slices and in dissociated cell cultures. We have previously shown dye coupling to be correlated with electrotoneic coupling in tissue culture. We have examined the modulation of electrotoneic coupling by hippocampal neurons by determining the extent of dye-coupling in culture and in slices in the presence of NMDA and C-kinase activators.

In control, intracellularly dye-coupled hippocampal neurons were dye coupled. Application of NMDA (10 nM) abolished dye-coupled (n=28) as did TPA (50 nM, n=21) a protein kinase C activator. Free dye coupled hippocampal neurons were dye coupled. Application of NMDA (10 nM) abolished dye-coupled (n=28) as did TPA (50 nM, n=21) a protein kinase C activator. Free dye coupled hippocampal neurons were dye coupled. Application of NMDA (10 nM) abolished dye-coupled (n=28) as did TPA (50 nM, n=21) a protein kinase C activator.

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369.4 FORSKOLIN ACCELERATES DESENSITIZATION OF 5-HT3 RECEPTORS IN MOUSE HIPPOCAMPUS AND NG108-15 CELLS. J.J. Fakel* & M.B. Jackson, Dept. of Biology, UCLA, Los Angeles, CA.

5-HT induces rapid currents in hippocampal neurons (Fakel et al., J. Neurosci. 8:1273) and NG108-15 cells (Christian et al., Brain Res. 472:261). Studies in NG108-15 cells show that this current is carried by sodium and potassium ions. IC50 205-930 (1 nM), a selective 5-HT3 antagonist, blocked these responses. ICS 205-930 (1 nM), a 5-HT3 receptor antagonist, blocked these responses. The magnitude of the response produced by 2-methyl-5-HT (50 µM) was 45.1 ± 12 (N=5) whereas 4α,8-Phorbol had no effect on GABA responses. Following washout of 2-methyl-5-HT (50 µM) the response recovered partially (81.5% ± 5.2, N=5, compared to 63.8 % ± 4.7 during 2-methyl-5-HT application in these 5 cells). These data indicate that activation of protein kinase C by phorbol 12-13 dibutyrate reduces GABA-A mediated chloride conductance. Taken together with previous findings (Neurosci. Abstr. 13, 279.7) it may be speculated that the GABA-A receptor is regulated by several protein kinases, as reported for the nicotinic ACh receptor (comp. Huganir and Greenberg, TIPS, 1997).

Supported by grants from NIH and Klingenstein Foundation.

Exposure to exogenous AD evokes a hyperpolarization probably caused by an increase in potassium conductance. In the present study, records were obtained from CA1 neurons of adult S.D. rats in vitro, employing single-electrode voltage clamp. We estimated the voltage and ionic sensitivity of AD effects. The amplitude and polarity of the AD evoked current varied with extracellular K (from 3.2, 6, 12.5, 25 mM) as predicted by the Nernst equation for a change in potassium permeability. I/V plots (membrane potential was altered from -120 to -50 mV at a rate of 1 mV/200 mS) showed an AD evoked increase in conductance that was voltage insensitive. Neither the addition of 1-5 mM CsCl nor 500 uM 4-AP to the perfusate affected the AD evoked hyperpolarization. However, the addition of 2 mM Ba reversibly blocked the AD responses.

Transient outward currents were also examined. InBa, a calcium dependent K current, was increased but Iapp was not. We conclude that AD evoked postsynaptic inhibition is mediated by a voltage insensitive increase in steady state potassium conductance and Iapp. The former is similar in its lack of voltage sensitivity and inactivation to a potassium conductance reduced by ACh (analogous to S current described in invertebrates) in CA1 neurons.

369.7 AN INCREASE IN POSTSYNAPTIC CALCIUM IS SUFFICIENT TO ENHANCE SYNAPTIC TRANSMISSION IN HIPPOCAMPUSS. J.A. Kauer, R.C. Malenka, R.S. Zucker, & R.A. Nicoll. UCSF, San Francisco, CA 94143 and Univ. Calif., Berkeley, CA 94720.

Using the hippocampal slice, we have performed two sets of experiments to define the importance of calcium in the induction of long-term potentiation (LTP). Intracellular electrodes were filled with the photolabile calcium chelator, Nitr5, loaded with calcium. Fluctuations in intracellular free calcium were analyzed by photolysis of Nitr5 that elevates calcium to the low micromolar range and measures anodic current on CA1 pyramidal cells for more than 20 minutes. No effect of light exposure was observed either on the extracellularly recorded population EPSP, or in control experiments using electrodes filled with Nitr5 which was not loaded with calcium. We demonstrate that the release of calcium was the factor responsible for the potentiation.

Pairing low-frequency stimuli to the presynaptic afferents with depolarization of the postsynaptic cell has been reported to produce LTP. If Ca*+ entry is necessary for the induction of LTP, one would expect that by holding a cell near the reversal potential for calcium, one could block LTP. Using Ca*-filled electrodes, pairing 10 afferent stimuli with a smaller depolarization produced a large and long-lasting potentiation. Taken together, these experiments demonstrate that calcium is both necessary and sufficient to trigger postsynaptic processes involved in synaptic potentiation.


Acetylcholine receptor (AChR) genes are differentially regulated during development. AChR are expressed on myotube cells which are nucleated and thus represent an interesting system in which to study nuclear activation and inactivation. We have used in situ hybridization with a series of cDNA probes to examine the distribution of AChR a subunit mRNA at the subcellular level. The a subunit genomic clone was obtained from Dr. Chang. Cells were hybridized with the labelled AChR a subunit probe and with labelled DNA probes for poly(U)RNA. The hydrids were detected by nuclear track emulsion which allowed a precise localization of the hybridized probe. The autoradiograms showed a diffuse distribution of silver grains throughout the myotube. Quantification of myotube nuclei revealed that 71% of nuclei actively express AChR a subunit mRNA at the subcellular level. Our results show that myotube nuclei are differentially activated for the expression of AChR a subunit mRNA and possibly other synaptic proteins.

369.6 MEMBRANE POTENTIAL FLUCTUATIONS DO NOT CONTRIBUTE TO SHORT-TERM SYNAPTIC PLASTICITY IN APHYLLONIC ACYCLUS. Daniel Gardner. Dep. of Physiology, Cornell University Medical College, New York NY 10021.

At identified inhibitory synapses in the Aplysia buccal ganglia, both duration and amplitude of synaptic currents are alterable by manipulating presynaptic activity, producing an enhancement of short-term synaptic plasticity. Recent theoretical reports have suggested modifications to Hebbian learning paradigms in which synaptic efficacy would be altered not by neuronal activity, but by changes in membrane conductance. In this study, we investigated whether variability-sensing learning rule which uses a biophysically plausible mechanism, I advance and test the hypothesis that changes in membrane potential (V_m) fluctuations themselves produce changes in postsynaptic current (PSC) strength. Fluctuations in I_m produced by imposed synaptic input might plausibly cause changes in I_m through a variability-sensing learning rule. This was tested by injecting varying variance (6 x 10^-4 V^2) noise over 1-50 Hz range) into identified identified voltage-clamped postsynaptic neurons, monitoring synaptic efficacy for 60-180 min, and comparing a common pre- and post-synaptic element. Different paradigms using three-cell networks evaluate non-specific and associative effects on individual synapses.

Non-associative effects of noise: PSCs were simultaneously recorded at 1 min intervals from the postsynaptic cells innervated by a common presynaptic neuron, with noise injected into one of the two between PSCs. Synaptic current amplitudes and durations varied in parallel in the two followed by a normalizing by control values suggests no non-associative effects of noise injection.

Associative effects of noise: In a complementary protocol, a postsynaptic neuron was alternately stimulated by two presynaptic cells, with noise injected during PSCs produced only one of the two. Neither PSC amplitude nor duration was consistently altered by associative pairing with noise.

I conclude that synaptic efficacy is affected by 1-2 h of noise simulating complex excitatory and inhibitory synaptic input from other pathways. Supported by ONR and AFOSR via N00014-89-K-0108 from ONR.

369.10 BIPHASIC CHANGES IN ACETYLCHOLINE BINDING TO POST-SYNAPTIC NICO TINE RECEPTORS BY BARBITURATES. B.A. Dedeken, R.L. Roch, L.L. Firestone and K.V. Miller. Dep. of Anesthesiology, University of California, San Francisco, CA 94143.

Preliminary data suggest that barbiturates have a biphasic effect on equilibrium [3H]acetylcholine ([3H]-ACh) binding to Torpedo postsynaptic acetylcholine receptors (AChR). To study this effect in more detail we devised an in vitro procedure to examine the distribution of AChR a subunit mRNA at the subcellular level. The a subunit genomic clone was obtained from Dr. Chang. Cells were hybridized with the labelled AChR a subunit, or with labelled DNA probes for poly(U)RNA. The hybrids were detected by nuclear track emulsion which allowed precise localization of the hybridized probe. AChR a subunit probe produced a diffuse distribution of silver grains throughout the myotube. Quantification of myotube nuclei revealed that 71% of nuclei actively express AChR a subunit mRNA at the subcellular level. Our results show that myotube nuclei are differentially activated for the expression of AChR a subunit mRNA and possibly other synaptic proteins.
The duration of glycine action at Mauthner cell inhibitory synapses is determined by diffusion, not Na-dependent uptake. N. Jilmus, D.S. Faber, and H.Kom. Dept. Physiol. SUNY at Buffalo, Buffalo, NY. 14214 and Dept. Biotechnologies, Pasteur Inst., Paris.

Recent evidence for interactions between adjacent glycineic synapses on the Mauthner(M)-cell suggested that diffusion, rather than an active uptake or inactivation mechanism, may account for the post synaptically determined. To test this idea we studied the effects of blocking Na-dependent glycine uptake on the kinetics of inhibitory responses evoked by stimulating the Mauthner(synaptic) and Mauthner postsynaptic. Removal of Na had no effect on the time course of the decay of evoked synaptic responses recorded in current mode. However, they were compared with controls obtained at the same membrane potential. The durations of the longer lasting (sloc vs 200 ms) conductance changes produced by glycine iontophoresis were unchanged. However, the magnitudes of these responses were enhanced in Li, with the glycine dose-response curves being shifted to the left by about 0.2 to 0.5 log units. This facilitation does not appear to be due to a change in glycine receptor properties, since the half coefficient, which averaged about 2. was unaltered by Li in 4 experiments. We conclude that the uptake of glycine does not influence the duration of glycine action following its release but may rather act to maintain a low concentration of transmitter in the synaptic cleft despite its high serum levels. (Supported in part by NS 21848)

The duration of glycine action at Mauthner cell inhibitory synapses is determined...
intrathecal arginine-vasopressin produces
peripheral cardiovascular responses in conscious and
anesthetized rats. A. Martinez-Arizala, J. B. Long,
and J. W. Holaday. Dept. of Medical Neurosciences, Wae reed

Prior studies in our laboratories suggested that the cardiovascular
cerid producers produced by intrathecal (i.t.) arg-
vasopressin releases peptides in conscious rats in
anesthetized rats. To further characterize the
to changes in the responses to i.t. AVP, S-D rats were implanted with PE
cathepters in the tail artery, the jugular vein, and the lumbar
subarachnoid space. Mean arterial pressure (MAP) and hindlimb
motor function were monitored following i.t. injections of AVP (0.01 nmol/kg) in conscious and anesthetized (urethane
and xylazine) rats had a pressor response to i.t. AVP and conscious
rats exhibited an acute hindlimb paralysis. The pressor effect of
i.t. AVP were mediatrd centrally at the V3 and recep-
ton were blocked by the i.t., but not the i.v. injection
the V3-receptor antagonist d(CH2)4[Tyr(Me)-AVP]. In addition,
the pressor effects of i.t. AVP were: a) blocked by
phenoxybenzamine and hexamethonium in the anesthetized,
but not in the conscious rats, and b) not blocked in conscious rats by
the angiotensin II antagonist [Sar1, Thr1]-angiotensin II. These
results indicate that sympathetically induced catecholaminergic mechanisms
mediator the rise in MAP produced by i.t. AVP in anesthetized rats,
but not in conscious rats.

370.7
HYPOTHALAMIC ANGIOTENSIN PEPTIDE RECEPTORS IN
ADRENAL-NEUROSECRETORY AND VASOPRESSIN-
DEFICIENT RATS. Sergio Guevara and Juan M. Sawada.
Laboratory of Clinical Science, National Institute of Mental Health.

Attrial natriuretic peptide (ANP) has receptors in
supra-optic and paraventricular nucleus of the
hypothalamus; these nuclei are implicated in the
regulation of the hormone release from the pituitary
gland. We have studied the physiological role of these
receptors by measuring ANP binding and ANP release in
anesthetized (ANP) and adrenalectomized (AXN) rats and in vasopressin-
deficient homozygous Brattleboro rats (DI) by receptor autoradiography according to Karibu et al.
(Brain Res. 408:311-39, 1987).

Following AXN, ANP binding sites were increased in both
supraoptic (37%>87) and antidiuretic hormone (ADH) for AX and
controls, respectively, P<0.001 and magnocellular
paraventricular nucleus (525±16±6 fmol/mg protein for AX and
controls, respectively, P<0.001). A similar increase
was observed in DI rats (262±19±2 fmol/mg protein in
SNH and 112±2±9 fmol/mg protein in AVP for AX and
controls, respectively, P<0.05). AXN did not alter
ANP binding in these nuclei.

These results support the hypothesis that hypothalamic
ANP receptors are mainly involved in the regulation of
vasopressin release.

370.9
IONIC BASIS FOR SUBSTANCE P EXCITATION IN TRIGEMINAL
ROOT GANGLION (TRG) NEURONS. J. Spigelman and E. Puli,
Dept. of Pharmacology & Therapeutics, Faculty of Medicine,
University of British Columbia, Vancouver, Canada, V6T 1W5.

The slow, depolarizing responses in the perikarya of TRG
neurons to substance P applications (Spigelman and Puli, Can J.
Physiol. Pharmacol., 1988) are of special interest because of possible involvement in the transmission of noxious impulses.

Initially it was proposed that substance P excitation in TRG neurons resulted from an increased Na+ conductance (gNa) in combination with a K+-conductance (gK). We now provide direct evidence using single electrode voltage clamp techniques for an activation of inward current during applications of substance P (2 μM) in the presence of 4-aminoopyridine (1 mM) and tetrodotoxin (10 μM). This current was also observed during internal Cs+-blockade of K+channels. Because changes in gNa/gK could result from actions on the Na+/K+ pump, the effects of extracellular norepinephrine were studied on the depolarizations evoked by substance P. The responses were drastically reduced in Mg2+-deficient solutions. The above observations imply that the ionic mechanism of substance P action on TRG neurons is unlike that proposed in central neurons.

Also, extracellular [Mg2+] may modulate the substance P responses at the level of peptide-receptor interaction.

Supported by a Medical Research Council grant to E. Puli and a Canadian Heart Foundation Research Traineeship to J. Spigelman.

370.10
SYNERGISTIC EFFECTS OF F-PREPTACHYKININ-DERIVED PEPTIDES
ON SALIVARY GLAND SECRETION. Y. Takeda and J.E. Krause, Department
of Anatomy & Neurobiology, Washington State University,
St. Louis, MO 63110

The preprotachykinin (PPT) precursors encoding Substance P (SP) and Neuropeptide K (NK) display a high degree of homology in regions that do not encode these tachykinins, and the precursors may be differentially processed post-translationally. These observations have led us to examine some biological responses to these peptides, and to examine whether they coordinate actions with SP or NK on their biological endpoints. We examined the activity of F-PFT-derived peptides on salivary gland responses in rats, and document potent effects of a neuropeptide K (NK) peptide amide, F-PPT(7-10)-peptide amide) on the salivation response as well as its synergism with SP in inducing salivary gland secretion. The rank order of potency of feline venous injection of SP and NK on salivation response was: SP > NK, and NK > SP, and others, including PPT(7-10)-peptide, were inactive. NK- induced responses occurred at lower doses than that of SP, and the maximal response was greater. Salivation responses induced by NK and NK-SP antagonized by the tachykinin antagonist (D-Pro2, D-Trp7,9-SP), but not by atropine. The potency of NK (X=100 μM) on the displacement of NK binding to the SP type receptor was 100-fold lower than that of SP (X=100 μM). When both NK and SP were co-injected for 10 min at subsalivary doses, these peptides greatly stimulated salivation secretion over a similar time course than either peptide alone, but the total response was some 3-fold greater than the sum total of individually infused NK and SP.

We conclude that NK is a potent tachykinin, and the synergistic effects of NK and SP on the salivation response may represent an important mechanism of tachykinin co-referential in tachykinin-elicited biological responses.

We treated rats chronically with the potent cholecystokinin (CCK) antagonist L-364,718 for a 14-day period at a dose of 1.5 mg/kg/day (CA Watson et al., Soc. Neurosci. Abstr., this meeting). In the treated animals (N=9), the spleens were uniformly and grossly enlarged as compared to vehicle-treated controls (N=9) and were distinguished by a diffuse scattering of collections of histiocytes resembling small, non-necrotizing granulomas in the red pulp area. The anorexia in treated animals showed a diminution in the size of the inlets of Langerhans. Other organs, including the exocrine pancreas, were grossly normal. Enlargement of the spleen was not observed in acutely-treated (1-day) rats. Our results strongly suggest that chronic blockade of peripheral CCK receptors with large doses of L-364,718 produces multiple pathological changes. The functional changes associated with these alterations are currently under investigation.

Supported in part by NB94595 (GRF), NS00149 (GRF), NS27871 (LRS).


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Supported in part by NB94595 (GRF), NS00149 (GRF), NS27871 (LRS).
CEREBRAL METABOLISM AND BLOOD FLOW II

BRAIN GLUCOSE METABOLISM DURING FASTING IN MAN STUDIED BY POSITRON EMISSION TOMOGRAPHY. L.J. Hofstetter, C. Redies, C. Benk, B.B. Marlies, A.C. Evans, P. Larrieu, S. Marron, P. Mager, M. Diksic, and A.M. Rakita.* (From: G. Karpati.) Montreal Neurological Institute and McGill Nutrition and Food Science Centre, McGill University, Montreal, Canada H3A 2B4

Positron emission tomography (PET) was used to study cerebral glucose and oxygen metabolism, blood flow, and blood volume in four hospitalised obese men (age 38 ± 6SD years, body mass index 36 ± 4 kg/m²). PET studies were carried out on a control diet (10-14 hours after previous meal), and during fasting.

Cerebral metabolic rate for glucose (CMRglc) and glucose tracer rate constants (Kf and Kt) were measured using [3H] labeled fluoro-deoxyglucose and dynamic imaging. Cerebral metabolic rate for oxygen, blood flow, blood volume, and oxygen extraction ratio were measured with [14O] labeled O2, CO2, and CO gases.

Regional CMRglc fell in 38-47% of control values (p < 0.002). The rate constant for tracer phosphorylation (Kt) fell to 42-57% of control values (p < 0.002). Both parameters decreased relatively uniformly throughout the brain. Regional blood-brain barrier tracer rate constants for tracer (Kf and Kt), metabolic rate for oxygen, blood flow, and blood volume were unchanged.

Supported by MRC grant SP-5, and fellowships from MRC, DFG (Re 616/1-1), Max-Planck Gesellschaft, and Juvenile Diabetes Foundation.

DOUBLE-LABEL DEOXYGLUCOSE METHOD APPLIED TO FERRET VISUAL CORTEX. C. Redies and M. Diksic.* Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4

The quantitative double-label deoxyglucose method (Redies et al., Neurosci.22:601,1987) allows the separation of glucose utilization patterns in mammalian brain elicted by two sequential sensory stimulations.

The method requires the knowledge of the rate constants for transfer of tracer across the blood-brain barrier (Kf and Kt), tracer phosphorylation (Kt), and loss of metabolized tracer (Kt), and of the lumped constant (LC).

These constants were determined for fluoro-deoxyglucose (FDG) and 2-deoxy-glucose (2-DG) in 18 ferrets killed between 2.5 and 180 min after tracer injection. Mean gray matter values for [14C]FDG are: Kf = 0.19ml/g/min, Kt = 0.28/min, and LC = 0.003/min; and for [3H]FDG: Kf = 0.21ml/g/min, Kt = 0.29/min, and Kt = 0.15/min. Results suggest that loss of metabolized tracer (Kt) occurs at a constant rate of 0.01/min for 180 min after injection. However, whether Kt is 0 or 0.01/min, has a negligible effect on calculating glucose utilization in conventional 45 min 2-DG experiments provided that the entire analysis including the determination of the LC is consistent. Assuming Kt = 0.01/min, the LC in ferret brain is 1.03±0.05SEM for FDG and 0.70±0.09 for 2-DG.

Double-label experiments in the ferret visual cortex show that the method provides layer-specific information on functional "columns" elicited by two different sequential visual stimulations in the same animal. Supported by MRC of Canada (Grant MA-10233).


The uptake and retention of radioactivity was measured in areas of rat brain at different times after i.v. injection of [14C]-2-deoxyglucose (2-DG) or [6-14C]-glucose (G) with nuclear emulsion autoradiography. In most brain regions the accumulation of radioactivity from the two compounds was similar using a 30 min survival period for G and a 45 min survival period for 2-DG. However, at those times, relatively more radioactivity was accumulated from 2-DG in stratum lacunosum-moleculare of hippocampus and layer 4 of isocortex. In contrast, relatively more radioactivity accumulated from 2-DG in stratum lacunosum-moleculare of hippocampus and layer 2 of piriform cortex. With G at 5 and 30 min survival periods, the distribution of radioactivity was identical except in layer 4 of isocortex, where more radioactivity was present at 5 min. With 2-DG at 5 and 45 min survival periods the relative and absolute amount of radioactivity was greater at 5 min compared to 45 min in the dentate gyrus, CA1 pyramidal cell layer of hippocampus, and layer 2 of piriform cortex. In other brain regions, the absolute and relative amount of radioactivity was similar or slightly greater at 45 min compared to 5 min. These results demonstrate that neuroanatomically selective loss of radioactivity occurs after injection of 2-DG during a 45 min survival period.


The chronic administration of the neurotoxin iminodipropionitrile (IDPN) to rats causes a persistent behavioral syndrome consisting of random circling, vertical head twitches, and hyperactivity. IDPN was given intraperitoneally in 0.5 mg/kg doses, twice weekly, over a period of 7 weeks. The chronic administration of IDPN in discrete brain areas was due to primary actions in the brain. We, therefore, used the 2-deoxy-2-[1-14C]glucose method to measure LCUs in paralysed (pallial muscles relaxed) and artificially ventilated rats treated with the aforementioned drugs.

Rats received i.p. injections of saline, cocaine (30 mg/kg) or IDPN (30 mg/kg) or i.v. injections of saline. LCUs were measured in the substantia nigra, substantia reticularis, and cerebellum. Amphetamine elevated LCUs in the same areas and also the anteroverentral thalamus, zona incerta, subthalamic nucleus, substantia nigra pars reticulata, subthalamic nucleus, zona incerta, anteroverentral thalamus, globus pallidus and caudate putamen. These effects were seen in paralysed rats, but not secondary to limb movements, but rather were primary central effects.


Twelve male Sprague-Dawley rats were assigned to one of two groups, receiving a single daily i.p. injection of saline or IDPN, respectively. One day after treatment, the percentage of the characteristic behavioral syndrome, LCU, was measured as previously described (Sokoloff et al., J. Neurochem. 1:197,1973). Statistical analysis showed that 40% decreases in LCU were found within the superficial and deep layers of the superior colliculus, interpeduncular nucleus, inferior colliculus, medial and lateral geniculate nuclei, and medial and lateral vestibular nuclei. No significant decreases were seen in the substantia nigra. The clausenoid injection of IDPN did not contribute to the phenomena of IDPN-induced, persistent spasmatic dystonias in rats.

Gelsamin, a mammalian acetylcholine receptor (AChR) component of muscle basal lamina (BL), is found on embryonic rat myotubes prior to innervation. It is not present in mature motorneurons at any age. Immunoperoxidase staining of muscle fibers or in adult muscle fibers, gelsamin becomes localized to the synaptic BL. In the Torpedo electric organ, factors activating acetylcholine, gelsamin affects AChR protein levels and localization. Both gelsamin and CSRP increased total receptor protein content significantly: CSRP, as reported previously by Fontaine et al., 1987. Together, they effected a 6-14 fold increase in AChR α-subunit mRNA levels, each had a significant effect on AChR α-subunit mRNA levels, measured in Northern blots with a cRNA probe for mouse AChR α-subunit mRNA kindly provided by Dr. Jim Patrick. 15 µg/ml purified Gelasmin induced a 3-4 fold increase in α-AChR mRNA levels; α-CSP increased a 3-4 fold increase (as previously reported by Fontaine et al., 1987). Together, they effected a 6-14 fold increase in AChR α-subunit mRNA levels. The effect of gelsamin and CSRP appears to be synergistic in these preliminary studies. Further studies are underway to determine whether this represents stabilization or induction of the mRNA. Supported by the NSF, NIH, MDA, Phoenix Foundation and Office of the Vicepresident for Research, U. Michigan.


MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES


A monoclonal antibody designated 473 was obtained from rats immunized with L2/HNK-1 positive glycoprotein fractions from adult mouse brain. In immunofluorescence double labeling experiments performed on cerebellar cultures of varying ages no overlap with neuronal markers was observed. The antibody stained subsets of immature astrocytes and oligodendrocytes. At earlier stages of culture, half of the 473 cells did not express any of the conventional markers. Metabolic labeling experiments with subsequent immunoprecipitation performed with glial monolayers cultures in vitro often incorporates sulfate, fucose, and methionine. It migrates at 102 kDa under non-reducing conditions and was recovered from detergent lysates and supernatants of labeled cultures. Material with similar properties could be demonstrated in G26-20 glial tumor cells. Digestion of immunoprecipitates obtained with 473 from G26-20 cells gave several fragments, two of which were recognized by polyclonal antisera to chondroitinase ABC and AC. The 473 antigen carried the L2/HNK-1 epitope, a carbohydrate common to several adhesive molecules. Functional properties of the 473 proteoglycan are currently being investigated.

372.7 MONOCLONAL ANTIBODIES AGAINST NGF-DIFFERENTIATED PC-12 CELLS RECOGNIZE CELL SURFACE ANTIGENS EXPRESSED IN A 1:1 (MOl/MOl) RATIO.

A library of monoclonal antibodies against cell surface antigens of NGF-differentiated PC-12 cells was prepared using spleen cells of mice injected intact PC-12 cells which were cultured with NGF for 6-8 days. Culture fluid of these hybridomas was screened by ELISA using the intact cells and Millitter filter system. Many monoclonal antibodies in the library recognize antigens which expressed in the PC-12 cells in different manner during NGF-treatment. Hippocampal tissue from 16 days fetal rat were dissociated with trypsin-treatment and cultured for 1-2 weeks. The culture was immunostained with the monoclonal antibodies. Several monoclonal antibodies recognized the cell surface antigens of the cultured neurons which were identified with antibodies against neurofilaments. Some antibodies seemed to recognize extracellular matrix and glycolipids. The monoclonal antibody library is a useful tool to study cell surface molecules and their functions not only in PC-12 cells but also in CNS neurons to differentiate.

372.8 ECOTO-PROTEIN KINASE AT THE SURFACE OF CNS NEURONS.


To demonstrate secretion of an Ecto-protein kinase activity in the CNS, we have utilized embryonic mesencephalic neuritides differentiated during maintenance for 15-21 days in vitro (DIV) in a chemically defined medium. Depolarization with 50 mM KCl induced a Ca2+-dependent release of ATP, and stimulation by 100 μM veratridine induced ATP secretion that was blocked by tetrodotoxin. Incubation of intact, attached neurons with [γ-32P]-ATP (added to the medium) resulted in labeling of surface phosphoproteins within 10-15 min. These proteins were not phosphorylated when intracellular ATP pools were labeled with equivalent amount of inorganic 32P. Phosphorylation of surface proteins with M.W. >50Kd was dependent on 1mM Mg2+ but not on Ca2+, whereas phosphorylation of surface proteins with M.W. of 39-42 Kd required 5mM Ca2+ and was independent of Ca2+, as we reported previously with synaptosomes (J. Neurochem., 30: 263, 1988). Phosphorylation by extracellular ATP of proteins co-migrating with N-CAMS was high during the period of rapid neurite extension (4-6 DIV) and declined after synaptogenesis and maturation (15-18 DIV) indicating that ecto-protein kinase activity in the CNS is developmentally regulated. Supported by AFSRS grant no. 88-0004.

372.9 BRAIN SPECTRIN(240/235A): A NOVEL ASTROCYTE SPECIFIC SPECTRIN ISOFORM.


We have previously demonstrated the existence of two distinct isoforms of spectrin in mammalian brain. Brain spectrin(240/235) found in neuronal axons and presynaptic terminals, and brain spectrin(240/235) located in neuronal cell bodies, dendrites, and postsynaptic terminals (Riedeker et al. 1980, Cell Biol. 102: 208). In this study we have isolated a panel of monoclonal antibodies which all react exclusively with the 240kDa or 235kDa subunits of brain spectrin on Western blots of total rat brain protein. Hybridoma supernatants of rat cerebellum utilizing 32 distinct monoclonal antibodies, yielded 13 antibodies which gave a staining pattern typical of brain spectrin(240/235) and 3 antibodies which detected brain spectrin(235/235, 1 antibody which detected both isoforms, and 15 antibodies which detected a novel isoform in astrocytes. Only a subset of rat cerebellum was localized in the soma and fibrous processes of astrocytes and in the distal processes of Bergmann glia, but was not present in the deep cerebellar layers. The novel rat brain spectrin with the astrocyte specific monoclonals under stringent conditions, yielded 240k and 235kD subunits in a 1:1 mol/mol ratio. We have discovered mammalian brain spectrin isoform as brain spectrin(240/235A).

372.10 SEX DIFFERENCES IN 67-COPPER UPTAKE BY HYPOTHALAMIC (HT) AND HIPPOCAMPAL (HIP) SLICES.


Cu is an essential trace metal that is highly concentrated in the brain, particularly in axonal terminals and secretory vesicles. A role for Cu in modulating neuronal function is supported by our previous findings that Cu metabolism is altered during development in cultures of hypothalamic (HT) and hippocampal (HIP) slices. Prostaglandin E2 stimulation of peptide release from HT tissue and that newly taken up 67-Cu is released by depolarization. We have recently determined that HT slices take up Cu by a saturable, ligand-specific process having an apparent Km (40 μM) in the operable conc. range of Cu. We addressed the question: Are there sex and/or regional differences in 67-Cu uptake in the brain? Saturation curves were established for 67-Cu uptake by HT and HIP slices obtained from rat brains. Males: Vmax for 67-Cu uptake (P<0.001) in HT that was higher in HT (850 μg 67 Cu/mg/min/mg P) and in HIP than that in HT (850 μg 67 Cu/mg/min/mg P) of intact males and castrated (14 days) led to an increase (P<0.001) in Vmax in both regions (850 μg 67 Cu/mg/min/mg P) in females. Vmax was similar in HIP and HT of pregnant females (650 μg 700 pmol/min/mg P) and it was reduced (P<0.01) by ovariectomy (19 days) only in the HT. There was no difference in the apparent Km in these tissues. Thus, there are sex and regional differences in number of Cu-carryer sites in the HT and HIP and gonadal steroids appear to modulate the process of Cu uptake in the brain.
372.11 GLUCOCORTICOID DECREASE GLUCOSE TRANSPORT IN CULTURED HIPPOCAMPAL NEURONS. HEIDI C. HUTCHISON, STEPHEN A. MANCINSKI AND ROBERT M. SAPOLSKY, Biology Department, Stanford University, Stanford, California, U.S.A. 94305

Glucocorticoids decrease glucose transport in hippocampal neurons, but the mechanism is not known. We have found that a small (20 ng/ml) concentration of dexamethasone decreases glucose transport in dissociated hippocampal neurons. In primary cultures prepared from 14 day old rat hippocampus, we have found that the decrease in glucose transport is a function of the concentration of dexamethasone and is saturable. This decrease in glucose transport is not due to a change in the number of glucose transporters in the plasma membrane. In addition, the decrease in glucose transport is not due to a change in the affinity of the transporter for glucose. In order to determine the mechanism by which glucocorticoids decrease glucose transport, we have examined the effects of dexamethasone on the kinetics of glucose transport. We have found that the half-time of glucose transport is increased by dexamethasone, suggesting that the transporter may be changing conformation. These results suggest that glucocorticoids may be involved in the regulation of glucose transport in hippocampal neurons.

372.12 CALCIOSOME(S) IN MAMMALIAN BRAIN. P. VOLGE, B.H. ALDERSON, P.A. FAZIO, E. ROBERTS AND M. MAIDOLESF, SPON: M.G. ANDERSON. Dept. of Physiology & Biophysics, UTMB, Galveston, TX 77550 AND C. Raffaele Scientific Inst., Univ. of Rome, Italy.

Recent immunocytochemical and biochemical results have provided direct evidence for the existence of a hitherto unrecognized organelle in mammalian brain, homologous to the sarcoplasmic reticulum (SR) of striated muscles, which seems to be the inositol 1,4,5-triphosphate (IP3) and Ca2+ releasing organelle named "calcosome" (Volge, P., et al., Proc. Natl. Acad. Sci., 85:1091-1095, 1988). In the liver and exocrine pancreas, as well as in two cell lines, proteins related to calsequestrin (CS) and Ca2+, ATPase(s), were shown by immunogold labeling to reside not in the endoplasmic reticulum or previously other identified cytoplasmic organelle, but in a population of vesicles and small vacuoles distributed throughout the cytoplasm. We have now data indicating that such vesicles present in rat major azonial branch of canine brain yields a membrane fraction that actively accumulates Ca2+, binds (32)Pi, releases Ca2+ upon addition of IP3, and contains a protein immunologically related to CS. Immunochemistry of cultured neurons indicates that CS-positive organelles are localized both in the soma and neurites. The calcosomes is therefore likely to exist in nerve cells. (Supported by NIH grant GM 40068-01.)
373.5

TEMPORAL POLE PROJECTIONS TO THE MAGNOCELLULAR MEDIAL DORSAL NUCLEUS (MDmc) OF THE RAT. G.L. Allen and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

Limbic system connections have been analyzed intensively in subprimate species using both anatomical and electrophysiological methods, but since experimental anatomical methods cannot be used in human brain, functional studies must be used to assess connectivity. Bauer and Benchard (ENG 373.5) studied connections between hippocampus (HP) and amygdala (AM) using stimulation of depth electrodes implanted in these structures to monitor epileptic activity in medically intractable patients who were candidates for surgical seizure therapy. They reported mean onset latencies of 18-19 msec between HP and AM. Studying a group of 31 patients with complex partial epilepsy, we delivered single-pulse high intensity stimulus 100 µsec in duration to each of five limbic electrodes in 12 patients from mesial and lateral structures, and to the slightly more differentiated proisocortical areas of the temporal lobe. Onset latencies to HP were shorter (7.1 ms) than the previous study while to mHP they were longer (24.8 ms), suggesting their different conduction velocities.

Comparison to other species must be on the basis of conduction velocities (CV), which have not previously been reported in human limbic sites, and therefore CVs were calculated for the connection between each stimulation and recording site. CVs ranged from 0.88 m/s in the AM to 3.4 m/sec in the perforant path connection from EC to mHP. This was followed closely by a 3.2 m CV in the longitudinal association connection from mHP to Amygdala. Our results are consistent with a trend toward slower rates of conduction on the higher phylogenetic scale, associated with a second trend of decreased fiber diameter and increased number of fibers in primates. NIH grant NS 02808.

373.6

CORTICAL PROJECTIONS TO ORBITOFRONTAL LIMBIC CORTEXES IN THE RHESUS MONKEY. Dept. of Health Sci. and Anatomy, Boston Univ. Sch. of Med., Boston, MA 02215.

Ipsilateral cortical projections to the least architectonically differentiated basal periallocortex, and to the slightly more differentiated perisylvian prefrontal areas were studied with the use of the retrograde transport of horseradish peroxidase (HRP). These areas, which exhibit an incipient laminar differentiation, received most of their projections from other limbic cortices. Most of the projections originated in nearby orbital and medial limbic prefrontal areas. HRP-labeled neurons were also found in temporal polar, perirhinal, and prorhinal areas. It appears that impulses from the limbic system may influence the activity of cerebellar regions which are known to project to vermal regions of the cerebellum. Thus, impulses from the limbic system may play a fundamental role in memory. (Supported by VA Medical Research funds.)

373.7

CONVERGENCE OF AFFERENTS FROM MEDIAFRONTAL CORTEX AND MAMMARY BODY IN MEDIAL PONTEAL NUCLEUS OF THE RAT. G.L. Allen and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The pontine nuclei receive afferent fibers from limbic regions of the cerebral cortex and from the hypothalamus. In order to provide a better understanding of functional links between the limbic system and the cerebellum a light and electron microscopic study of limbic system connections with precerbellar relay nuclei has been undertaken. Stereotaxic injections of 5% WGA-HRP were made into cortical hypothalamic and stem thalamic nuclei. After three days, rats were perfused with a buffered aldehyde fixative. For light microscopy, frozen sections were incubated in TMB followed by a buffered DAB-H2O2. For electron microscopy, vibratome sections were incubated in TMB followed by a buffered aldehyde fixative. For light microscopy, frozen sections were incubated in TMB followed by a buffered DAB-H2O2.

Axon terminals in the medial pontine nuclei which originated from the medial prefrontal cortex and the mamillary body contained small round vesicles and formed asymmetric synapses with small diameter dendrites.

The present results demonstrate that afferents from the prefrontal cortex and the mamillary body with similar morphological characteristics and synaptic organization converge on neurons in the medial pontine nuclei which in turn project to vermal regions of the cerebellum. Thus, impulses from the limbic system may influence the activity of cerebellar regions which are known to be involved in certain autonomic functions.

Supported by MRC of Canada.

373.8

NEUROPHYSIOLOGICAL MEASURES OF HUMAN LIMBIC SYSTEM CONNECTIONS. C.L. Wilson, M. Fugger-Anderson, K.R. Pregenzer, S.L. Kien, H. Baak, Dept. of Neurosurgery, University of Munich, Germany, D-8000 Munich, FRG; UCLA School of Medicine, Los Angeles, CA 90024.

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373.9


Different projections from rostral temporal lobe cortices to the magnocellular medial dorsal nucleus (MDmc) were studied in the monkey (Macaca mulatta) with intracortical injections of horseradish peroxidase (HRP) and tritiated amino acids (TAA). TAA injections into pyriform allocortex or the transitional neocortical fields of the temporal pole (periallocortex and proisocortex) produced discrete zones of axonal terminal degeneration in MDmc characterized by bursts of coarse label surrounding neuronal perikarya and their proximal dendrites. Although the same anterograde field was observed when HRP was injected into the transitional cortices, perikarya within the terminal clusters were not retrogradely labeled. Label was not transported to MDmc when injections were made into the superior or middle temporal gyri, and thus restricted to the isocortex. These findings indicate that a non-reciprocal corticothalamic pathway to MDmc originates in the phylogenetically older districts of the temporal pole. This pathway constitutes an additional temporal route to MDmc paralleling those contributed by the amygdala and the hippocampal formation. The conduction of limbic sensory information directly to the basal periallocortex may play a fundamental role in memory. (Supported by VA Medical Research funds.)

373.10


In series of sections cut at various thicknesses in the sagittal, frontal and horizontal plane through the human interpeduncular nucleus several subnuclei were delineated on account of lipofuscin pigmentarchitectonic and cytoarchitectonict criteria. Previous investigations revealed that nerve cell types in the human interpeduncular nucleus can reliably be characterized by their lipofuscin pigment pattern. The interpeduncular nucleus extends from the level of the caudal pole of the nucleus to the level of the frontal pole of the locus coeruleus. It is about 8 mm long, 2 mm broad and 1 mm high. A medial subnucleus extends from the frontal pole of the caudal pole and contains 2 types of small non-pigmented neurons and 3 types of sparsely pigmented neurons. In the frontal half, additionally a scattered population of richly pigmented neurons occurs. Within the frontal subnucleus, small sparsely pigmented and non-pigmented neurons are intermingled with large non-pigmented nerve cells showing distinct Nissl bodies. At the frontal level of the pons, a small lateral cell group can be outlined harbouring small neurons with numerous faintly stained lipofuscin granules. Caudally, this subnucleus is replaced by group of medium-sized neurons with numerous lipofuscin granules distributed between the elongated Nissl bodies. Supported by the Deutsche Forschungsgemeinschaft.
UNPUNISHED RESPONDING. The results argue against these predictions. Administration of chlordiazepoxide (10 mg/kg) with two replications.

AFTER a week's recovery, animals were retested on the unpunished responding. The results appear to mimic the effects of anxiolytic agents. Thus, during the first set of sessions at these currents septal stimulation produced increased responding in the punished periods whereas MFB stimulation did not. After several replications and raising of current levels in the MFB group, some of these animals displayed increased punished responding as well.


Resting behavior in the unpunished and signaled punished periods, each 2 min in duration, were alternated and cycled through twice. The punished period was signaled by tone and every PA 19010 shock was accompanied by 3.0-6.0 ms footshock. The number of licks was recorded throughout the 8 min session. After a stable baseline was achieved, rats were iontophoresed with bipolar stimulating electrodes in the lateral nucleus of the septum and in the MFB.

INITIAL EXTRACELLULAR ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CHOLINEURONIC (Ch-ACHN-positive) neurons of the medial septum/diagonal band (MS/DB) region of guinea pigs can be distinguished from non-Ch neurons by intracellular recording in vitro (Griffith and Matthews, Neurosci. Lett. 71:169, 1988).

Prominent features of the MS/DB neurons include: 1) long duration action potentials (AP), 2) slow firing rate when depolarized by current pulses, 3) presence of an inward Ca2+-sensitive current resulting in a shoulder on the repolarization phase of the AP and 4) presence of 5-H Ca2+ and 6) an inward Cd2+sensitive Ca2+ conductance resulting in a long duration post-spike hyperpolarization. Extracellular unit recordings from the same slice preparation yield extracellular representations of three of these four intracellular characteristics. Thus a subpopulation of MS/DB neurons (13 of 54) demonstrated: 1) long duration AP's (3.1 ± 0.27 ms versus 1.7 ± 0.13 ms for other neurons), 2) maximum firing rates when driven by microiontophoresed glutamate (12-17 Hz versus up to 120 Hz for other neurons), 3) multiphasic AP shape with significant shortening of the AP duration, 4) a Ca2+-specific K+ conductance, 5) a 0.7 ms decrease versus 0.1 ± 0.08 ms decrease for other neurons), and it is concluded that at least some Ch neurons of the MS/DB are distinguished from non-Ch neurons with extracellular recording techniques.


Animals were trained in a conflict test in which they drank for 2 min during unpunished segments which were alternated with signaled punished segments. The punished periods, signaled by tone, resulted in mild footshock (0.3-0.6 mA, 1 second duration) for every fifth lick. The number of licks was recorded for all unpunished segments throughout the 8 min session. Animals were trained to a stable baseline of suppression during the signaled punished periods and then administered chloridiazepoxide (10 mg/kg) with two replications.

Approximately half the animals showed some release of suppression during the signaled, punished periods after lesioning. Administration of chloridiazepoxide produced a large release of punished behavior with no effect on unpunished responding. The results argue against these proposed antagonistic areas as primary sites of action of the benzodiazepines.


Numerous anatomical studies have shown that the neocortex is a major target of the basal forebrain. However, the action of basal forebrain afferents on neocortex has not been described. This study presents the first in vivo characterization of synaptic responses of medial cortex to basal forebrain stimulation. Our sample of responsive neurons included 72 pyramidal cells and two fast-spiking, putatively nonpyramidal cells. The predominant synaptic response of cortical neurons was inhibition (55.4%). Reciprocal connections between recording and stimulating sites was indicated by a high incidence of antidromic action potentials (71.6%). Orthodromic potentials and antidromic spikes were generally observed in ipsilateral layer V neurons. The fast-spiking cells exhibited similar electrophysiological characteristics to previously described inhibitory interneurons, however, one produced an IPSP and both produced antidromic spikes following stimulation (differential band of Broca).Supported by NIH grant NS 23074.

INITIAL EXTRACELLULAR ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CHOLINEURONIC (Ch-ACHN-positive) neurons of the medial septum/diagonal band (MS/DB) region of guinea pigs can be distinguished from non-Ch neurons by intracellular recording in vitro (Griffith and Matthews, Neurosci. Lett. 71:169, 1988).

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In this study we measured the diurnal variations in density and affinity of β-adrenergic receptors in cortical and hypothalamic membranes from C3H/HeN and C57BL/6 mice, using 3H-DP and 3H-DHA as ligands in separate studies carried out over a 2 year period. The ages of subjects ranged from 18-90 years, with a binodal distribution, having subjects predominantly in young and old groups. Mean ages (± SE; N = number of subjects) were: DHA old gp: 63.8±9.5 (N=35), young gp: 23.4±2.9 (N=21); ICYP old gp: 70.3±3.9 (N=24), young gp: 29.5±2.1 (N=20). Potential subjects were excluded for physical or psychiatric illness. Lysates of hypothalamic membranes were prepared from freshly drawn venous blood according to a modification of the method of Brodde et al. (Life Sci 29:189, 1981), and in binding assays with either DHA or ICYP. Scatchard analysis gave estimates of binding maxima (Bmax) and antagonist affinity (Kd). In both studies, increases in Bmax, with no change in Kd, were found in the older groups. Values for Bmax were: DHA old gp: 64.8±3.9, young gp: 48.6±2.4; ICYP old gp: 81±17.7, young gp: 47.2±5.6. The increases in Bmax were highly significant (for DHA, F1,35 = 8.79, p<0.01; for ICYP, F: 1.14 = 19.06, p<0.001). Thus, in these preparations, the binding of beta antagonists to lysates of hypothalamus was increased with age. Supported by NIA Aging Grant # K07AG020300 and McNeil Pharmaceutical, Inc.
The bundles generated tension while soaked in high K+ solution and were done at room temperature (20-22°C). Epinephrine and Isoproterenol muscle of Rana pipiens. Normal solution was (πM): NaCl 117.5, KCl 2.5, CaCl2 1.8. pH was adjusted to 7.4 with Imidazole chloride. Experiments of adrenergic and serotonin binding sites in striatum and cortex had not been previously reported. Using standard radioligand filtration binding assays, performing dose-response studies of a minimum of 22 points each, replicated, and using LiGANG to resolve the data, ye observed that MPP+ inhibited ligand binding at (H)PSP (HSP) binding sites in postmortem human cortex (spiperone) and at cortical (H)1H2J14,304 (α2-adrenergic) binding sites. Shown below are the Kd for MPP+ (the Kd are given in parentheses) at each of the radioligand affinity states detected.

\[
\begin{array}{lll}
\text{Radioligand} & \text{High Affinity} & \text{Low Affinity} \\
\text{[3H]MPP+ Cortex} & 1.8 \times 10^5 & 6.6 \times 10^5 \\
\text{[3H]MPP+ Putamen} & 6.4 \times 10^6 & 9.3 \times 10^5 \\
\text{[3H]UK14,304} & 2.9 \times 10^6 & 4.8 \times 10^5 \\
\text{[3H]Norsip} & 5.5 \times 10^6 & 2.1 \times 10^5 \\
\end{array}
\]

These findings suggest that MPP+ has potentially complex interactions with neurotransmitter systems.


We have shown previously that cAMP in the extracellular fluid of the brain can be detected using an implanted microdialysis probe (Trans. Am. Soc. Neurochem. 19:159, 1988; Fase J., 2:1810, 1988). In the present studies we have further developed this technique into a method for studying the function of noradrenergic and other cAMP-linked receptors in the intact brain. Urethane-anesthetized rats were implanted in the frontal cortex with microdialysis probes placed with various agents for various periods. cAMP in the perfusate (dialysate) was assayed by RIA (sens. 2 fmol). In agreement with previous studies in brain slices, norepinephrine (NE), isoproterenol (ISO) and adenosine (10^-10^-3 M) were found to induce dose-dependent increases in in vivo cAMP levels. The response to NE was blocked by the beta antagonist, timolol. Infusion of the alpha receptor agonist, 6-fluorodopamine, led to a potentiation of the responses to ISO and adenosine. Prolonged infusion of ISO caused a progressive desensitization of the cAMP response and the latter effect was partially reduced by prior treatment with corticosteroids. The results are in accordance with previous in vitro studies and indicate that the microdialysis-cAMP method can be used to study the function of noradrenergic and other receptors in the brain in vivo. (Supported by MH22768 and MH08616).

**ADRENAL MEDULLATION II**, A. C. Anderson and J. Pickens* F.D.R. V.A. Hospital, Montrose, N.Y. 10548 and Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio.

MPP+, an oxidative product of MPTP, is a selective dopaminergic neurotoxin. MPP+ interactions with the dopamine reuptake system have been well documented. The effect of MPP+ on dopamine, α2-adrenergic and serotonin binding sites in striatum and cortex had not been previously reported. Using standard radioligand filtration binding assays, performing dose-response studies of a minimum of 22 points each, replicated, and using LiGANG to resolve the data, ye observed that MPP+ inhibited ligand binding at (H)PSP (HSP) binding sites in postmortem human cortex (spiperone) and at cortical (H)1H2J14,304 (α2-adrenergic) binding sites. Shown below are the Kd for MPP+ (the Kd are given in parentheses) at each of the radioligand affinity states detected.

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The use of the dopamine agonist 2-aminopropylcaine (APM), L-αseryl-L-phenylalanine methyl ester (APM) represents an additional dietary source of phenylalanine (Phe), although plasma levels of this aminoacid were not measured for a long period of time after APM consumption. Since Phe and its metabolite tyrosine (Tyr) may influence some CNS neurotransmitter systems, it was of interest to investigate the effects of chronic APM consumption on brain aminergic activity. Male Sprague-Dawley rats were given 50 or 500 mg/kg APM per day in drinking water for 30 days. Cerebral cortex and striatum were excised for determination of amine levels and for study of binding kinetics at several receptors: adrenergic α1 (prazosin) and β (clonidine), dopaminergic D1 (SCH 23390) and D2 (spiperone), and serotoninergic 5HT2 (ketanserin). Kd and Bmax values were estimated from 6 parallel determinations (2 animals pooled per determination), each consisting of 6 ligand concentrations. We found no significant changes in binding parameters for any of the receptors studied. In addition, no significant differences were found in brain dopamine, serotonin, and norepinephrine levels in our present experimental design. Our findings of no change in binding affinity and density lead to the conclusion that, in rats, APM treatment is unlikely to affect catecholaminergic or serotoninergic activity.

**Progestrone Modulation of α and β Adrenergic Receptor Interactions in Hypothalamic Slices**, N. Petitti and A.M. Erdogan. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Norepinephrine (NE)-stimulated cyclic AMP (cAMP) accumulation in hypothalamic and preoptic area slices was monitored to examine the effects of progesterone (P) on brain adrenergic and serotoninergic receptor function. Treatment decreased NE-induced slice cAMP accumulation. This effect was dependent on prior estrogen exposure and was independent of increases in progestosterone activity or decreases in adrenochrome or melatonin. In all slices cAMP levels were elevated by isoproterenol, a β receptor agonist, but not by α agonists (epinephrine) or α agonists (clonidine). However, in slices from estrogen plus P-treated rats, clonidine potentiated the effect of isoproterenol on cAMP formation whereas phenylephrine did not. In contrast, phenylephrine but not clonidine enhanced isoproterenol-induced cAMP accumulation in slices from rats receiving only estrogen. In P-exposed slices, NE-stimulated cAMP accumulation was completely antagonized only by a combination of both β (propranolol) and α (yohimbine) antagonists. The data suggest that treatment of estrogen-primed rats (1) depresses NE-stimulated cAMP accumulation in hypothalamic and preoptic area slices, (2) decreases or eliminates α1 receptor function, (3) promotes an α2 receptor-mediated inhibition of cAMP synthesis, and (4) potentiates an α2 receptor augmentation of β receptor stimulation of adenylate cyclase.

**ADRENAL MEDULLATION II**, A. C. Anderson and J. Pickens* F.D.R. V.A. Hospital, Montrose, N.Y. 10548

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DOPAMINE RECEPTOR MODULATION AND REGULATION

THURSDAY AM

375.1


375.2

COMPARISON OF BEHAVIORAL AND BIOCHEMICAL CONSEQUENCES OF TWO DISTINCT MODELS OF DOPAMINERGIC DENERVATION SUPERSENSITIVITY. B. E. Milisen and R. P. Malman. Univ. of North Carolina Curriculum in Toxicology and Biological Sciences Research Center, Chapel Hill, NC 27599.

Behavioral and biochemical endpoints of two different models of dopaminergic denervation supersensitivity were compared in order to examine possible mechanisms of supersensitivity. Denervation of central dopamine afferents resulting in severe dopamine depletion was induced by unilateral (IC) administration of 6-hydroxydopamine (6-OHDA) (200 μg) or bilateral infusion of 6-OHDA into the substantia nigra (8 μg/side). Low-dose agonist challenge of recovered rats lesioned in the nigra elicited a number of behaviors not seen in sham lesioned control rats, including self-grooming, intense grooming and ardent grooming of the cage floor. IC-lesioned rats responded to agonist challenge by explosive locomotor activity and rearing. Receptor binding studies performed on striatal membranes of both lesioned and control rats revealed no change in the number of binding sites (B_max) or the dissociation constant (K_d) of either D1 or D2 receptors for either lesioned group compared to controls. Behavioral function of D1 receptors was evaluated by measurement of dopamine-sensitive adenylate cyclase in striatal membrane preparations. Differences in dopamine-stimulated adenylate cyclase activity due to lesion were slight, indicating additional mechanisms are involved in mediating behavioral supersensitivity. Supported by PHS Grants ES01104 and MH40537.

375.3

TEMPORAL CHANGES IN STRIATAL D1 DOPAMINE RECEPTOR DISTRIBUTION AFTER DOPAMINE DESTRUCTION. T. Delorenzo1 and M. A. Ariano. University of Vermont College of Medicine, Burlington, VT 05405.

A comparison of the morphochemical distribution of striatal D1 dopamine binding sites, 3 days to 20 weeks following unilateral infusion of 6-OHDA into the substantia nigra, has been performed. Autoradiographic localization of the specific D1 antagonist ligands &-apo-DSCH 23930 or &-DSCH 23982 was assessed in relation to cyclic AMP immunocytochemically characterized striatal neurons. [Br. Res. 467]204,1988). The extent of dopamine depletion in the striatum was determined biochemically or morphologically. The present study examined the ability of long-acting dopamine (LORG) to block acute and chronic HAL-induced decreases in specific binding sites in the substantia nigra, globus pallidus, and caudate-putamen. The extent of dopamine depletion in the striatum was measured before and after chronic administration of agonists. Treatment with quinpirole or quinpirole plus SKF-38393 resulted in a desensitization of the D2-mediated depression of dopamine levels which was consistent with results of the biochemical measurements. None of the treatments produced desensitization of the D1-mediated oral dyskinesia which is also consistent with the biochemical results. (Supported by USPHS grant GM 34781)

375.4

CCK ANTAGONIST LORGULMIDE BLOCKS ACUTE AND CHRONIC HALOPERIDOL-INDUCED EFFECTS ON DA NEURONS. L. L. Jiang and J. P. Zanger. Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

The present study examined the ability of lorgulmid to block the acute and chronic HAL-induced reductions in D1 dopamine binding sites on midbrain DA cells. LORG is a more potent and selective CCK antagonist than proglumide (PROG) in pancreas. Male Sprague-Dawley rats were treated with HAL (5 mg/kg/day). Standard extracellular single unit recording techniques were used to determine the number of spontaneously active DA cells in anatomically defined A9 and A10 regions. Similar to the results obtained with PROG, i.v. LORG reversed the HAL-induced reduction of DA cells/track in both A9 and A10. Moreover, microinjection of LORG (0.8 mg/m1, but not 0.1 ml) directly into the nucleus accumbens (mNAC), an area containing a high density of GABA/beta nerve terminals and receptors, dose-dependently reversed HAL-induced effect. LORG injected into the lateral NaC or the caudate-putamen was without effect. In addition, i.v. or microinjection of LORG into the mNAC also reversed acute HAL-induced firing rate increase of both A10 and A9 DA cells. These results strongly suggest that CCK receptors in the mNAC form an important link for maintaining HAL-induced effect on midbrain DA neurons and CCK is involved in the therapeutic action of haloperidol in rats. (Supported by USPHS Grants MH-41440, MH-4196 and MH-00378 to R.Y.W.)

375.5

ENHANCED RESPONSES OF NUCLEUS ACCUMBENS (NAc) NEURONS TO DOPAMINE (DA) D1 AND D2 RECEPTOR AGONISTS FOLLOWING 6 MONTHS TREATMENT WITH ANTIPSYCHOTIC DRUGS (APDs). Xi-Ti Hu and Ren X. Wang. Dept. of Psychiatry & Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

The present study was designed to test the hypothesis that long-term treatment with antipsychotic drugs (APDs) could be the result of enhanced dopamine (DA) receptor sensitivity following long-term APD treatment. The present study was designed to test the hypothesis. Male Sprague-Dawley rats were treated with either haloperidol (HAL, 1 mg/kg/day), clozapine (CLOZ, 25 mg/kg/day) or water via their drinking bottles for 6 months. The technique of single-unit recording and microiontophoresis were used. At low ejection currents (1-5 nA), both SKF and LY facilitated the CCK-induced inhibition of glutamate- and glycine- induced responses in both HAL and CLOZ-treated rats (with or without drug withdrawal period) compared to control. Dose response curves for both SKF and LY were shifted to the left in HAL- and CLOZ-treated rats. In contrast, no significant difference in the sensitivity of CCK neurons to iontophoresed SKF-38393 (a D1 receptor agonist) in control, HAL and CLOZ groups, although many of these APD-treated rats showed spontaneous oral movements. HPLC analysis of the extracellular HPLC analysis of the extracellular dopamine (DA) receptor agonist in control, HAL and CLOZ groups, although many of these APD-treated rats showed spontaneous oral movements. HPLC analysis of the extracellular dopamine (DA) was equivocal. This is in agreement with the observations that DA agonist-induced changes in striatal cAMP and inositol phosphates were not different in control and APD-treated groups. They concluded with the results from studies showing that compared to controls, there was a significant increase of D2 receptor binding sites in the Cpu following chronic APD treatment. In conclusion, our results do not support the view that TD is the result of DA receptor supersensitivity. (Supported by USPHS Grants MH-41440, MH-4196 and MH-00378 to R.Y.W. and MH-00378 to R.Y.W.)

375.6

THE SENSITIVITY OF RAT CAUDATE-PUTAMEN (Cpu) NEURONS TO D1 AND D2 RECEPTOR AGONISTS ARE NOT CHANGED BY LONG TERM TREATMENT WITH ANTIPSYCHOTIC DRUGS (APDs). R. X. Wang, L. H. Jiang and J. P. Zanger. Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794.

dopamine was effected in rats by intracaudate injection of HAL or CLOZ into the lateral NaC or the caudate-putamen was without effect. In addition, i.v. or microinjection of LORG into the mNAC also reversed acute HAL-induced firing rate increase of both A10 and A9 DA cells. These results strongly suggest that CCK receptors in the mNAC form an important link for maintaining HAL-induced effect on midbrain DA neurons and CCK is involved in the therapeutic action of haloperidol in rats. (Supported by USPHS Grants MH-41440, MH-4196 and MH-00378 to R.Y.W.)

The present study was designed to test the hypothesis that long-term treatment with antipsychotic drugs (APDs) could be the result of enhanced dopamine (DA) receptor sensitivity following long-term APD treatment. The present study was designed to test the hypothesis. Male Sprague-Dawley rats were treated with either haloperidol (HAL, 1 mg/kg/day), clozapine (CLOZ, 25 mg/kg/day) or water via their drinking bottles for 6 months. The technique of single-unit recording and microiontophoresis were used. At low ejection currents (1-5 nA), both SKF and LY facilitated the CCK-induced inhibition of glutamate- and glycine-induced responses in both HAL and CLOZ-treated rats (with or without drug withdrawal period) compared to control. Dose-response curves for both SKF and LY were shifted to the left in HAL- and CLOZ-treated rats. In contrast, no significant difference in the sensitivity of CCK neurons to iontophoresed SKF-38393 (a D1 receptor agonist) in control, HAL and CLOZ groups, although many of these APD-treated rats showed spontaneous oral movements. HPLC analysis of the extracellular dopamine (DA) receptor agonist in control, HAL and CLOZ groups, although many of these APD-treated rats showed spontaneous oral movements. HPLC analysis of the extracellular dopamine (DA) was equivocal. This is in agreement with the observations that DA agonist-induced changes in striatal cAMP and inositol phosphates were not different in control and APD-treated groups. They concluded with the results from studies showing that compared to controls, there was a significant increase of D2 receptor binding sites in the Cpu following chronic APD treatment. In conclusion, our results do not support the view that TD is the result of DA receptor supersensitivity. (Supported by USPHS Grants MH-41440, MH-4196 and MH-00378 to R.Y.W. and MH-00378 to R.Y.W.)
DOPAMINE RECEPTOR MODULATION AND REGULATION

927

b in d in g o f [3H]SCH-23390 to striatal membranes in v i t r o . The numbers of striatal D-1 receptors were challenged by 17 levels. When PLG (1.0 mg/kg/ip) was co-administered effects of SCH-23390. Critical in the development of striatal D-1 receptors, QUILLEN-DISHNER College of Medicine, East Tennessee State Univ., Johnson City, TN 37614. While the effects of receptor antagonists on the development of striatal dopamine D-2 receptors have been studied by several groups, ontogenic actions of D-1 receptor agonists have not been determined. Bats were treated from the day of birth and once every day for 32 successive days with the selective D-1 antagonist, SCH-23390 (10 mg/kg/d, ip). In a binding assay of Schulz et al. (J. Neurochem. 65: 1601, 1995), it was found that chronic SCH-23390 treatment during postnatal development resulted in a 25-40% reduction in the D-1 binding of [%]H]SCH-23390 to striatal membranes in vitro at 5, 8, and 12 weeks from birth. These changes are associated with a 50-75% reduction in BMAX, as assessed at 5 weeks. When 12 wk old rats with reduced numbers of striatal D-1 receptors were challenged by 17 levels of SCH-23390 (0.30 ml/100 g ip) and receptors up-regulated 3-fold, up to 75% of control levels. When PLG (1.0 mg/kg/d, ip) was co-administered with SCH-23390, the ontogenic impairment of striatal D-1 receptors was totally attenuated. These findings demonstrate that the postnatal period is critical for the development of the D-1 and that PLG protects against the adverse ontogenic effects of SCH-23390.

928


Previous reports have suggested that chronic treatment with high potency D1 and D2 dopamine agonists inhibit the hydrolysis of phosphoinositides in striatum. We sought to replicate these findings and investigate whether long-term treatments in haloperidol (HD) or the high potency D2 dopamine clozapine (CLZ) modified these effects. Male Sprague-Dawley rats were treated for 12 months with HDL, 1 mg/kg/day; or CLZ 25 mg/kg/day. After a 60 min preincubation in Krebs-Ringer-Bicarbonate buffer (KRB), striatal slices were labeled for 2 hr with 0.7 µM [3H]inositol. In addition, slices from rats treated with 100 µM of labeled slices were added to KRB containing 10 mM Li+ and test drugs (final volume = 0.5 ml). After 30 min, the incubations were terminated by the addition of 1.5 ml CHCl3/MeOH and inositol phosphates were separated by ion exchange chromatography. Carbachol (0.5 mM) increased the accumulation of IP by 240% in both treatment groups as well as the matched controls. IP2 and IP3 were increased 28-44% and 8-30%, respectively. Quinpirole, a selective D2 agonist, had no effect on IP or IP3 levels at concentrations of 0.1-10 µM. Paradoxically, IP3 was increased minimally in controls, 15% in the CLZ group. This effect was not dose-dependent and did not reach statistical significance. These findings conflict with the results by Piazza et al. (Soc. Neurosci., Abs., Vol. 13, 213, 1987) and may represent an effect of aging. The results are discussed in relation to previous presentations of studies of DA receptor density, neuronal firing rate and adenosine cyclic activity in these chronically treated rats.

Cyclo(Leu-Gly) (CLG) PREVENTS NIGROSTRIATAL Dopamine (DA) SUPERSENSITIVITY (SS) IN MICE. C. Fields*, M. Lee*, M. Gonzalez*, P. Racz, and T. Hoff, Science 203:1133, 1979). Because Previous reports have suggested that muscarinic agents stimulate and D2 dopamine receptors. The only models in which the SS is permanent have been shown never been shown whether CLG can reverse an already established behavioral SS to DA agonists. We now document such a reversal in the OXH model. OXH rats showed significantly more stereotypic sniffing after apomorphine (APO: 0.45 mg/kg, ip) in a locomotor activity (APO: 0.15 mg/kg). CLG (100 mg/day for 8 days, 8 mg/kg, sc) reversed these SS responses. We conclude that the OXH model is useful for modeling the permanent or irreversible aspect of DA SS; [2] CLG may be useful in the treatment of DA-related diseases. (Supported in part by the Tourette Syndrome Assn & VA grants to JZF and RFR)
Evidence exists for the presence of dopamine (DA) autoreceptors on chromaffin cells. DA agonists inhibited nicotine-evoked catecholamine release from adrenal chromaffin cells and this inhibitory effect was reversed by DA antagonists and would make an ideal system to study the intracellular signals that are coupled to such receptors. PC12 cells are derived from transformed rat pheochromocytoma PC12 cells, possibly by increasing IP_3 formation and by activating calcium/calmodulin-dependent protein kinase and protein kinase C. Supported by USPHS grants NS01927 and NS01999.
CATECHOLAMINES IV

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376.5
CATECHOLAMINE (CA) SECRETION MEDIATED BY A OBUANIN RECEPTOR IN THE CEREBRAL CORTEX, HIPPOCAMPUS, SEPARATING STRUCTURAL AND FUNCTIONAL ASPECTS. H.B. HIEBER* (SPON: I. De Andreis.) Hypertension-Endocirc, Br., Nat. Heart, Lung, and Blood Institute, and Lab. of Cell Biology and Genetics, Nat. Inst. of Diseases, Digestive and Kidney Diseases, Bethesda, MD 20920.

We studied the secretion of CA from isolated bovine adrenal chromaffin cells in culture, in order to elucidate the possible correlation between binding and transport of CA, with blockade of Na+, K+-ATPase activity and the presence of the cardiac glycoside receptor as indicated by the presence of its bovine binding sites. Carbonic anhydrase release CA (up to 80% of total content) in a dose-dependent manner with an order of potency sympathetin > obuain > acetyldigoxin > digoxin.

The following experiment was performed to ascertain the possible role of Na+, K+-ATPase activity and the presence of the cardiac glycoside receptor as indicated by the presence of its bovine binding sites. Carbonic anhydrase release CA (up to 80% of total content) in a dose-dependent manner with an order of potency sympathetin > obuain > acetyldigoxin > digoxin.

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Rats will readily self-administer cocaine directly into the medial prefrontal cortex (mPFC), indicating that this site may be important in mediating the rewarding effects of this popular drug of abuse. Surprisingly, little is known regarding the electrophysiological effects of cocaine in this area. In the present studies, extracellular single-cell recording and microiontophoretic techniques were employed to characterize the effects of cocaine in the dopaminergic terminal area within the mPFC (layers V & VI). Our studies indicate that both cocaine (0.01 M) and dopamine (0.1 M) inhibit spiking activity in mPFC neurons. Cocaine (0.01 M), administered in a similar fashion, exerted no such inhibitory effect. The inhibitory effects of cocaine and dopamine were blocked by co-iontophoresis of the D2 dopamine antagonist sulpiride (0.05 M, 16 nA). At higher ejection currents, (>32 nA) local anesthetic-like effects (rate inhibition accompanied by spike amplitude reduction) were observed with both cocaine and procaine. Intravenous administration of cocaine also partially inhibited the firing of mPFC neurons. Present studies are investigating the mechanisms underlying the inhibitory effects of cocaine within the mPFC.
737.3

ENHANCED SENSITIVITY OF SUBSTANTIA NIGRA DOPAMINE (DA) CELL AUTORECEPTORS FOLLOWING PARTIAL DA DEPLETIONS IN RATS. Michael L. Puck and Anthony A. Grace. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA, 15260.

Studies have shown that 2 weeks after lesions causing greater than 85% depletion of striatal DA, there is development of striatal DA receptor supersensitivity. However, it has not yet been established whether the DA cell autoreceptor also undergoes changes in sensitivity. The present study investigated this problem using single-unit recording techniques and identified substantia nigra dopamine neurons in 6-hydroxydopamine lesioned and control rats. The relative sensitivity of lesioned and control rats was estimated by their response to the administration of dopamine (DOP) and amphetamine (AMPH). DOP was given intravenously to rats in each group in dose-responsive fashion. Preliminary results suggest a significant increase in sensitivity to DOP. In the group receiving DOP and AMPH was given intravenously to rats in each group in dose-responsive fashion. Preliminary results suggest a significant increase in sensitivity to DOP.

737.4

EFFECT OF AMPHETAMINE ON SINGLE UNIT ACTIVITY IN THE OLFACTORY TUBERCLE OF FERrets. Frank F. Schall, Michael J. Miller, and Andre Ferron. Centre de recherche en sciences neurologiques (Department of Neurology), Université de Montréal, Montréal, Canada.

To investigate the effect of dopamine (DA) uptake blockade on neuronal responsiveness to this catecholamine, we compared the duration of the response to DA before and after the administration of GBR 12909 (DA uptake enhancer, 10 μg/kg, i.v.) or DES (DA uptake blocker, 500 μg/kg, i.v.). The duration of inhibition induced by iontophoretic applications of DA (40 nA x 60 s) was assessed by the RT90 method, i.e., by measuring the time between the end of DA application and the return to a neuronal firing rate equal to or less than 90% of the preinjection frequency. In control rats, the duration of DA responses were significantly enhanced by GBR 12909 in the prefrontal cortex (87 ± 11 s), the striatum (110 ± 60 s) as well as in the nucleus accumbens (52 ± 20 s), but markedly reduced in the anterior cingulate cortex (NAC) (56 ± 15 s). Furthermore, DA depletions and/or desensitizations, using either MPTP or 6-OHDA, produced a decrease in the duration of the responses to DA (61 and 54 s, respectively) in the NAC, comparable to that observed after GBR administration. A possible explanation for the present results is that in the NAC, under normal circumstances, the released DA is transported back into the presynaptic terminals and in this way induces a further DA release, thus favouring a longer duration of DA effects. Such a positive feedback mechanism could play a key role in the regulation of the activity of DA neurons by modulating the homeostatic control of DA release. This control mechanism of DA release may be a particular feature of the mesocortical dopaminergic fibers projecting to the NAC and thus be involved in higher integration cortical functions.

737.5


We have previously reported that basal dopamine (DA) receptor stimulation is necessary for (enables) functional expression of postsynaptic D2 DA receptor stimulation. To explore further the nature of this enabling phenomenon, we used iontophoretic techniques to determine whether the DI receptor responsible for enabling is coupled to a diacylglycerol (DG) generating pathway. Single-unit recordings were obtained from the nucleus accumbens (NAC) of chloral hydrate anesthetized male rats. Dose-related DI DA agonists exerting varying efficacies (+27-70% efficacy as compared to DA) at stimulating cAMP formation were compared (SKF 57567, SKF 38193, SKF 38194). Preliminary results with the compounds indicate no differences in their ability to inhibit the firing of NAC neurons, suggesting that activation of AC may not be involved in the inhibitory effects of DI agonists on NAC neurons, or that only a small activation (≤245) is required to produce such effects. In addition, studies, iontophoretic application of dibutyryl-cAMP or 8-bromo-cAMP failed to potentiate (enable) quinpirole-induced inhibition of NAC neurons in DA-depleted rats. The present results suggest that the mechanism by which DI receptor stimulation enables DI mediated inhibition of NAC activity may not involve activation of AC.

737.6

EFFECT OF ACUTE AND CHRONIC TREATMENT WITH SCH 23390 ON THE SPONTANEOUS ACTIVITY IN VIVO OF DI TESTING DA NEURONS. Louis E. Esposito* and B.S. Bunney. (Spons: David J. Barker). Dept. of Psychiat., New Haven Med., New Haven, CT.

In the albinor rat, chronic treatment with typical antipsychotic drugs (APD) induces a marked decrease in the number of spontaneously active A9 and A10 dopamine (DA) neurons due to the development of depolarization block (DB). It has been shown that SCH 23390, a selective blocker of D-1 DA receptors, has a biochemical and behavioral profile consistent with potential antipsychotic activity. In the present study, we investigated the effect of acute and chronic administration of SCH 23390 on the spontaneous activity of DA neurons, both in the A9 and A10 areas, in order to elucidate the role of this drug in the DB produced by APD. The two groups of rats were given an acute subcutaneous (s.c.) injection of SCH 23390 (0.5 and 1.0 mg/kg). In four separate chronic experiments, SCH 23390 was administered repeatedly for 21 days either s.c. (0.5 and 1 mg/kg) or orally (5 and 10 mg/kg). Active A9 and A10 DA neurons were counted by passing an electrode through a stereotactically defined block of tissue that could be reproductively located from animal to animal. No changes in the number of spontaneously active DA neurons were found either after acute or chronic treatment with SCH 23390. D-1 receptor blockade, therefore, would not appear to be involved in the induction of DB by typical APD.

737.7

WHOLE-CELL RECORDINGS FROM NEURONS ACUTELY ISOLATED FROM THE RAT SUBSTANTIA NIGRA ZONA COMPACTA. N.L. Silva, C.M. Pochura, J. Hennessy-Mullen, and J. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

Neurons were acutely isolated from the substantia nigra zona compacta of 1-3 week old rats by enzymatic and mechanical dissociation according to the methods of Kay and Wong (J. Neurosci. Methods 16:227-238, 1986). This procedure yielded numerous verbeau and fusiform shaped cell bodies (≈500 μm in diameter) and numerous ovoid and fusiform shaped cell bodies with 2-5 truncated processes. Their electrophysiological properties were investigated immediately following dissociation using whole-cell patch recording techniques. Unfortunately, these neurons exhibited spontaneous activity, with spikes with spike heights of 60-100 mV. Current clamp recordings revealed that these neurons maintained resting membrane potentials between -50-60 mV and input resistances of 400,000 Ω. Following the application of TTX spiking was eliminated and evidence of an inward rectification was observed. Interestingly, this conductance has not been observed in embryonic cultured dopamine neurons. A variety of inward and outward currents were examined under voltage clamp. Two sustained outward currents could be distinguished by their sensitivity to TEA or Co and Cd. A transient outward current which was inactivated at depolarized potentials and to Co and Cd was also observed. The remaining inward current was observed to be sensitive to Cu and Cd. A transient outward current which was inactivated at depolarized potentials and to Co and Cd was also observed. The remaining inward current was observed to be sensitive to Cu and Cd.
CATECHOLAMINES: ELECTROPHYSIOLOGY II


Dysfunctional neurons may underlie the schizophrenic psychosis. We have used retrograde labelling to identify these cells in midbrain cell cultures. The nucleus accumbens, as a tract pugs was injected with rhodamine-coated latex microspheres (Katz et al., 1984), the ventral tegmental area was isolated after 3 days, dissociated (Kay and Wong, 1986), and cultured, and studied after 1 week. Microsphere-labelled cells in vitro had the morphology of dopamine neurons; they were large, multipolar, and had eccentric nuclei (cf. Berger et al., 1982). Tyrosine hydroxylase and catecholamine staining revealed that at least 85% of labelled cells were dopaminergic (cf. Swanson, 1982). Intrastomnic Lucifer Yellow injection showed that the cells had grown extensive processes, with variabilities likely to be presynaptic sites; often a single neighboring cell also fluoresced, suggestive of gap junctional connections. Microsphere-labelled cells showed a number of the properties characteristic of midbrain dopamine neurons: (i) broad action potentials, measuring 1.6 ± 0.2 msec, from peak to peak (cf. ii) hyperpolarizing afterpotentials, (iii) anomalous rectification, (iv) low-threshold depolarizations often triggering spike generation, and (v) responsiveness to exogenous dopamine. Adding physiological criteria, living dopamine neurons can be unequivocally identified. Studying synapses formed by these neurons should contribute to understanding of the function of central dopamine systems.

377.11 RESPONSE OF MEDIAL ZONA INCERTA NEURONS TO VENTROMEDIAL HYPOTHALAMUS (VMH) STIMULATION AND APPLICATION OF DOPAMINE IN VITRO. M.J. Eaton and R.L. Moss. Dept. of Physiol., Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235

The zona incerta (ZI), located in the subthalamic region of the diencephalon, contains dopaminergic neurons which are believed to have autoreceptors. The ZI has been implicated in such diverse functions as drinking behavior, gonadotropin secretion and sexual behavior. Anatomical studies have shown afferent input into the medial ZI from the VMH, another brain region which plays a role in these functions. The present study was designed to investigate the relationship between the effect of different input from the VMH and the microproeure of application of dopamine on the electrical activity of neurons in the medial ZI. Transverse sections through the ZI were obtained from Sprague-Dawley female rats and maintained in a slice chamber with continuous perfusion of warmed, oxygenated artificial cerebrospinal fluid. Entaralcal cell recordings were made from 347 neurons located in the medial ZI. The effect of microproeure application of 0.1M dopamine HCL on the neuronal activity, as well as the orthorhoadmic response to VMH stimulation, was determined for each neuron.

Dopamine Response

<table>
<thead>
<tr>
<th>Type</th>
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</thead>
<tbody>
<tr>
<td>Orthodromic (OD)</td>
<td>None</td>
</tr>
<tr>
<td>Response type</td>
<td>OD+</td>
</tr>
<tr>
<td># of neurons</td>
<td>OD+</td>
</tr>
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These data indicate a large, predominantly excitatory (OD+), input from the VMH to the medial ZI. Many of the cells in this region are dopaminergic and are believed to be capable of autoregulation. The results show a large proportion of the cells which are orthorhoadmically excited by stimulation are also inhibited by dopamine, suggesting that VMH neurons project to and synapse on dopaminergic neurons in the medial ZI. Supported by HD09986-V.

377.10 THE EFFECTS OF DOPAMINE ON CENTRAL AMYGDALA NEURONS, IN VITRO. M. C. Schloss, E. Asprodinì* and P. Shinbrot-Gallagher. Dept. Pharmacology, Univ. Texas Medical Branch, Galveston, TX 77550.

The central nucleus of the amygdala receives a vast amount of monoaminergic input and has one of the highest concentrations of dopamine (DA) in forebrain structures. Using 500 micron extracellular slices from adult male rats and intracellular recording techniques, we characterized the effects of superfusing one microan DA and apomorphine (Ap0) on the central amygdala neurons. DA hyperpolarized the membrane potential, decreased resistance and increased conductance in 41% of the cells tested (N=34) and 50% tested with Ap0. The voltage-current (V-I) relationship between control and DA had an intercept at -110 mV suggesting hyperpolarization was mediated by an increase in potassium conductance. In another 40% of the neurons DA superfusion produced hyperpolarization (10/34) or no change (-3/34) in resting membrane potential, increased conductance and decreased resistance (Ap0 252). The V-I relationship between control and DA did not intersect but rather displayed a parallel shift. In a small percentage of neurons (12%) DA depolarized the membrane and decreased conductance. According to their active properties there are two types of neurons in the central amygdala (N=100). The majority of neurons do not accommodate, approximately 15% accommodate despite stimulation with large cathodal pulses. Both neuronal types have prominent after-hyperpolarizations that are reduced by DA.


Recent neuropharmacological work has implicated thalamic dopaminergic neurons in the genesis of certain psychiatric conditions (Oke & Adams, Schiz. Bull.13:589,1987). We have investigated the effects of dopamine and Haloperidol, a dopamine antagonist, on the electrophysiological properties of Guinea Pig thalamic neurons in vitro. In particular, voltage-dependent spikes and voltage-dependent calcium currents were investigated in thalamic slices using current clamp and single electrode voltage clamp techniques. In this preparation DA, at concentrations of 200µM, increased the peak amplitude of the transient calcium current by 20-40%. Two other types of experiments have shed light regarding the mechanism of this effact. (1) Haloperidol, which is known to be a blocker of D2 dopamine receptors, decreased the peak amplitude of this current by 44 ± 7% at concentrations of 5-10µM. (2) Forskolin (which increases intracellular cAMP levels) also decreased the peak amplitude of this current by 42.5 ± 4.5% at concentrations of 10µM. These experiments show that the effect of the DA is not mediated via D1 receptors, which, by being coupled to adenylate cyclase, should increase the intracellular levels of cAMP. The action of these drugs on the low threshold calcium conductance suggests that a class of psychotic events may be related to altered intrinsic electroresponsiveness in thalamic and other central neurons. Supported by NIH grant NS13742 and a Fellowship to E. G-B from the Generalitat Valenciana.
ASSOCIATION OF TWO PERSEUS TOXIN SENSITIVE G-PROTEINS WITH THE D2 DOPAMINE RECEPTOR FROM MOUSE

**378.2**

**ASSOCIATION OF TWO PERSEUS TOXIN SENSITIVE G-PROTEINS WITH THE D2 DOPAMINE RECEPTOR FROM MOUSE**

**K. Harada**, **M. Tokoro**, **K. Tsuchida**, **M. Goldstein**, and **E. Mellor**

Research Laboratories of Schering AG (Berkelin-Bergkamen), N.Y. Univ. Med. Ctr., New York, N.Y. 10016, and E.

We have previously reported that EM 23,448 has a selective DA action on presynaptic and postsynaptic postsynaptic DA receptors (Goldstein et al., J. Neural Transm. 70:193, 1987). As a continuation of our studies we have examined the effects of EM 49,980, a 5-hydroxy derivative of EM 23,448, on DA autoreceptors regulating synthesis and release of DA. EM 49,980 dose-dependently (0.01-0.5 mg/kg, i.p.) inhibited 6-hydroxynorepinephrine (2 mg/kg, i.v.) and 6-hydroxydopamine (2 mg/kg, i.v.) induced striatal dopa synthesis. Following partial inactivation of DA receptors with 6-hydroxynorepinephrine-2-dihydroxy-1,2-diarylbenzylamine-1 (5 mg/kg, s.c.) and 6-hydroxydopamine (5 mg/kg, s.c.), EM 49,980 was shifted to the right, suggesting the presence of a receptor reserve at the DA autoreceptors. The electrically stimulated release of [3H]-DA from striatal slices was inhibited by EM 49,980 at high concentrations of 0.01-1.0M. However, in the presence of the DA uptake inhibitor nisoxetine (10 mg), EM 49,980 did not inhibit the electrically stimulated release of [3H]-DA. The increased concentration of DA in the synapse in the presence of nisoxetine may diminish the DA autoreceptor activity of EM 49,980. The results of this study show that EM 49,980 is a partial DA autoreceptor with its potency dependent on the synaptic concentration of DA. Supported by NIH grants NS-06010-21 and NS 25619.

**DOPAMINE RECEPTORS III**

**378.1**

**DOPAMINE RECEPTORS III**

R.S. Salah, D.M. Kuhn and M.P. Galloway

Department of Pharmacology and Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

In studying the signal transduction mechanism of the D2 dopamine receptor it has been demonstrated that the solubilized receptor exhibits high and low affinity states for dopaminergic agonists. G protein nucleotides and pertussis toxin can convert the receptor from a high affinity state to a low one. A D2 receptor preparation from bovine striatum, partially purified by affinity chromatography on a haloglycerol dependent, exhibited agonist stimulated cGMP activity. ([32]P]-cAMP synthesis by pertussis toxin of this receptor preparation resulted in the specific labeling of 39 and 41 kDa, in SDS-PAGE. Association of these G proteins with the receptor was specifically inhibited by Gpp(NH)P. Protocytotic fragmentation, immunoprecipitation and immunoblot analysis of these G proteins, indicated that the 39 and 41 kDa protein bands are analogous to brain Gl and Go respectively. These experiments demonstrate that two distinct pertussis toxin sensitive Gproteins are functionally associated with bovine striatum D2 dopamine receptor.

**378.3**

**STIMULATION OF RAT STRIATAL GT PASE BY ENOLIZES IN VITRO**

D.J. Holtz, B.M. Marchant, and M.J. McCormack

Research Laboratories of Schering AG (Berkelin-Bergkamen), Neuropharmacology, Millersville 170-176, 100 Berlin 65, F.R.G.

Many ergolines, with pharmacologically defined DA agonist properties, are also shown to be potent inhibitors of the neuronal enzyme tyrosine hydroxylase (TH). Several ergolines, specifically mescaline, ergotamine and dihydroergotamine, inhibited the in situ activity and phosphorylation of tyrosine hydroxylase. Using several ergolines to probe the partial agonist and antagonist properties of these ergolines, different ergolines inhibited in different ways, i.e., the maximum inhibition of basal synthesis.

**378.4**

**DOPAMINE (DA) AGONIST PROFILE AT SYNTHESIS MODULATING DA AUTORECEPTORS (AR) IN RAT STRIATAL SLICES**

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Utilizing striatal brain slices, we have developed an in vitro model to study synthesis modulating DA ARs. For example, Salah et al. (this meeting) used this preparation to demonstrate that stimulation of DA ARs directly modulates the phosphorylation of tyrosine hydroxylase. Using several DA agonists from different chemical classes, we found that potencies (EC50's) obtained in vitro generally parallel those obtained in other AR models. Indeed, compounds such as (-)-(3R)-fluoxetine and (+)-mianserin, previously defined as DA AR agonists, exhibit typical agonist character under basal conditions whereas their partial agonist character becomes evident after increasing extracellular DA. Notably, however, are differences among agonists in their intrinsic activity, i.e., the maximum inhibition of basal synthesis. Catechol containing compounds such as APO, DA, 67-OH-a-methyltetralin and SKF-38393 produce >90% inhibition whereas ergolines such as quipazine decrease basal rates by 50% maximum. Although direct inhibition of TH by catechols may contribute to the net effect, the mono-OH a-methyltetralin (j=7) and 5-OH-DPAT also exhibit >90% inhibition. This characteristic, combined with well-defined stereoechemistry of the OH-DPATs, makes these AR agonists useful to define the in vitro AR model. Support: USPHS DA-4120, MH-41227, Mich. Dept. Mental Health.

**378.5**

**NEUROSTRIATAL DOPAMINE AUTORECEPTORS INHIBIT TRANSMITTER SYNTHESIS BY DECREASING THE PHOSPHORYLATION OF TYROSINE HYDROXYLASE.**

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Research Laboratories of Schering AG (Berkelin-Bergkamen), Neuropharmacology, Millersville 170-176, 100 Berlin 65, F.R.G.

The in situ activity and phosphorylation of tyrosine hydroxylase (TH) was studied in rat striatal slices. TH activity was monitored by measuring the accumulation of dihydroxyphenylalanine (DOPA) in the presence of a decarboxylase inhibitor. TH phosphorylation was assayed by immunoprecipitation of TH with rabbit anti-TH serum. Incubation of slices in the presence of K+ (50 mM), forskolin (10 uM), dibutyryl-cAMP (1 mM), or 8-bromo cGMP (100 uM) results in an increase in the activity and phosphorylation of TH. The dopamine (DA) autoreceptor agonist pergolide (2-10 uM) inhibits both basal and K+ stimulated phosphorylation of TH. This change is accompanied by parallel changes in TH activity. The effect of pergolide was greatly diminished when slices were preincubated with the DA antagonist eticlopride. In slices prepared from rats pretreated with intrastriatal injections of pergolide, the inhibitory effect of pergolide was greatly diminished. These results are consistent with the hypotheses that 1) nerve terminal DA autoreceptors in the rat striatum are negatively coupled to adenylate cyclase, and 2) inhibitory effects of DA autoreceptor agonists on TH activity are due to a decrease in cAMP-mediated phosphorylation. Supported by NIH 09673 (RSS), DA 04120 (MPP), and the State of Michigan.

**378.6**

**STIMULATION OF DOPAMINE (DA) AUTORECEPTORS BY EM 49,980.**

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Department of Pharmacology and Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

In studying the signal transduction mechanism of the D2 dopamine receptor it has been demonstrated that the solubilized receptor exhibits high and low affinity states for dopaminergic agonists. G protein nucleotides and pertussis toxin can convert the receptor from a high affinity state to a low one. A D2 receptor preparation from bovine striatum, partially purified by affinity chromatography on a haloglycerol dependent, exhibited agonist stimulated cGMP activity. ([32]P]-cAMP synthesis by pertussis toxin of this receptor preparation resulted in the specific labeling of 39 and 41 kDa, in SDS-PAGE. Association of these G proteins with the receptor was specifically inhibited by Gpp(NH)P. Protocytotic fragmentation, immunoprecipitation and immunoblot analysis of these G proteins, indicated that the 39 and 41 kDa protein bands are analogous to brain Gl and Go respectively. These experiments demonstrate that two distinct pertussis toxin sensitive Gproteins are functionally associated with bovine striatum D2 dopamine receptor.
378.7 COMPARISON OF RECEPTOR RESERVE AT DOPAMINE (DA) AUTORECEPTORS IN VIVO AND STRIATAL, NUCLEAR AND OLFACTORY TUBERCLES. K. Bohmaker,* T. Puts,* M. Goldstein and E. Miller (SPON: J.C. Miller). Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

D2 DA autoreceptors in rat striatum which medicate local negative feedback inhibition of neurotransmitter synthesis show a larger receptor reserve for (+)-N-methyl-6-phenoxy-2-(1H)-indolinophtho-(2,1-b)azepine (NP) (Miller et al., Mol. Pharmacol. 31:592-598, 1987), whereas postsynaptic striatal D2 receptors do not (Miller et al., this meeting). It was therefore of interest to determine whether DA autoreceptors in other brain regions (e.g. mesolimbic areas) also possess spare receptors for the full agonist NP. Dose-response curves were obtained for NP reserve for γ-hydroxybutyrate (GLB)-induced L-DOPA accumulation in control and EEDQ (6 mg/kg)-treated rats. In control rats, the ED50 for NP was 1.24, 0.44 and 0.34 μg/kg in striatum (STR), nucleus accumbens (NAS) and olfactory tubercle (OT), respectively. In EEDQ-treated rats the corresponding values were 3.36, 1.44 and 3.65 μg/kg.

After EEDQ treatment, NP maximally reversed the GLB-induced increase in L-DOPA levels by 57, 79 and 100% in STR, NAS and OT, respectively. Analysis of the results indicated that the DA autoreceptors in all three areas display non-linear receptor occupancy vs. response relationships for NP; mesolimbic autoreceptors appear to have an even larger receptor reserve than striatal autoreceptors. Supported in part by NIH grants NS 23618, MH 27171 and MH 35976.

378.9 CHARACTERIZATION OF THE BINDING OF SCH 39166 TO D-1 RECEPTORS IN VIVO. R.D. McQuade, R.A. Harvey* and E. Chipkin. Schering Corp., 60 Orange St., Bloomfield, NJ 07003.

SCH39166 ((-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo[2,1b]azepine), a new D1 selective antagonist, has been analyzed for binding to D-1 receptors, in vivo. Rats receive a single s.c. injection of 125I-SCH39820 (SCH39820) in the presence of increasing doses of SCH39166; after 1 h, the striata and cerebellum are removed and counted. SCH39166 displaces the specific binding of 125I-SCH39840, with an IC50 of 11.4 ± 3.9 nM. This value is nearly 4 x higher than that observed for SCH39820 (3 nM), and correlates well with the relative affinities of the molecules in vitro. However, SCH32390 inhibits the striatal binding of 125I-SCH38840, whereas 1 μM of SCH39166 displaces only 75% of the bound ligand. The remaining bound is higher for SCH32390 than for SCH39166 (90% of the initial binding), but lower for SCH39166 than for SCH32390, but 10 μM of ketanserin, a 5-HT2 antagonist, significantly decreases the residual binding of 125I-SCH38840. Thus, SCH39166, unlike SCH32390, does not interact with 5-HT2 receptors in vivo. This study shows that SCH39166 selectively labels striatal D-1 receptors in vivo.

378.10 THE SELECTIVE D1 ANTAGONIST, SCH 32390 (SCH) DOES NOT INDUCE EXTRAPYRAMIDAL SIDE EFFECTS (EPS) IN CEBUS MONKEYS. V.L. Coffin*, M.B. Latranyi* and R.E. Chipkin. Schering Corp., 60 Orange St., Bloomfield, NJ 07003.

The following study evaluated the EPS liability of a D1 antagonist SCH vs. the predominantly D2 selective antagonist, haloperidol (HAL). Cebus monkeys were given either vehicle (NPD); SCH (10 mg/kg po) or HAL (0.3 mg/kg po) once a week for 3 weeks. SCH was 250 fold less potent at D1 receptors (Ki=2nM) while SCH was 250 fold less potent at D2 receptors (Ki=512nM). In vivo, SCH inhibited conditioned avoidance responding in both rats (MEDD=10 mg/kg) and Cebus monkeys (15 mg/kg) with a long duration of action; this is indicative of potential anti-psychotic activity. These in vivo effects are probably not due to D2 receptor blockade since SCH did not cause hyperprolactinemia in rats and did not block dopamine-induced emesis in dogs. SCH increased dopamine turnover but only to levels 50-70% greater than baseline; in contrast, haloperidol increased levels by >300%. In summary, SCH is a specific D1 antagonist both in vitro and in vivo with potential, long-lasting anti-CAR effects and is a potential novel anti-psychotic.


We report A-69024 is an antagonist of the D1, but not the D2, dopamine receptor. Radioligand binding studies using rat striatum shows A-69024 (HBR) to have an apparent affinity toward the D1 receptor of 12.5 μM (identifying using 125I]-SCH 23390), and an apparent affinity toward the D2 receptor > 10 μM (identifying using [3H]-U-66444B). A-69024 (HBR) is an antagonist of the D1 receptor (Ki = 48.3 μM) and the D2 receptor in rat intermediate lobe (Ki = 728 μM); A-69024 (HCR) is an antagonist of rat striatum D1 receptor (Ki = 12.5 nM). A-69024 (HBR) has a ED50 of 6.5 mg/kg sc, with a 3 fold locomotor activity and stereotypy, and SKF 38393- (but not LY 15555-) induced rotation in 6-OHDA lesioned animals; however, at this dose there was not increase serum prolactin levels or potential DOPA accumulation in rats pretreated with the DOPA decarboxylase inhibitor NSD 1015. Thus, A-69024 can discriminate between the D1 and D2 receptors and, therefore, may be a useful research tool complementing observations made with other D1 antagonists and agonists.


U-64644B (BC) was reported to have presynaptic dopamine agonist activities (VonVoiglander et al., 1987). Some closely related analogs are found to be either potent dopamine agonists or antagonists. The following compounds are emetic in dogs: Ia, Ic, Id, Ie and If. Ia and Ic are B-2 emetic, but block apomorphine (D1)-induced emesis. In rats trained to avoid shock in a shuttle box, Ia, Ic, Id, Ie and If facilitate avoidance and increase movement between trials. Ia and If block avoidance and antagonize Apo-induced stereotypy. Neither compound produces catalepsy. In rhesus monkeys trained to discriminate 0.1 mg/kg of Apo from saline, both compounds block the discriminative stimulus effect of Apo. The dopamine agonist/antagonist activities in this series, therefore, depend critically on the position of the hydroxy group and the amino substitution.
DOPAMINE RECEPTORS III

Potentially useful model for investigation of the neural substrate for respiratory rhythm generation.

Supported by grants MRC RMA-9719 and CPSID.

378.13


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NO-112 and NO-756 are new potent D1 antagonists. In vitro, NO-112 and NO-756 exhibit a Ki for the D1 receptor of 0.2 nM and inhibit dopamine-stimulated adenylate cyclase with a Ki of 2.5 nM. Corresponding values for SCH 23390 were 0.2 and 40 nM, respectively. NO-112 and NO-756 have also high affinity for the 5-HT receptors (Ki values of 18 and 4 nM, respectively). The iodine-ted analog of NO-756, i.e. NO-673, had similar affinity for both D1 and 5-HT receptors (Ki = 3.6 and 7.3 nM, respectively). In accordance, 125-I-NO-673 labelled in vitro and in vivo both D1 and 5-HT receptors.

In conclusion, NO-112 and NO-756 are members of a new series of benzazepines with high affinity for the D1 receptor, but as compared to SCH 23390 with relatively higher affinity for the 5-HT receptor and dopamine-stimulated adenylate cyclase. The latter characteristics equivalent to some extent those of clozapine i.e. NO-112 and NO-756 may have atypical neuroleptic characteristics like clozapine.

378.15

ELECTROPHYSIOLOGICAL AND METABOLIC EFFECTS OF (+)-AJ76, A SELECTIVE ANTAGONIST OF DOPAMINE AUTO-RECEPTORS. W.E. Hoffmann, J.T. Lum, and M.F. Piercey, The Upjohn Company, Kalamazoo, MI 49001.

Svensson et al. report that (+)-AJ76 and (+)-UH 232 are selective antagonists of dopamine (DA) autoreceptors (Naunyn Schmiedebergs 334:234, 1986). We describe here 1) the antagonism of DA autoreceptor stimulation in substantia nigra pars compacta (SNPC), and 2) the use of 2-deoxyglucose (2-DG) autoradiography to map the neuroanatomical distribution of (+)-AJ76 effects. DA neurons of chloral hydrate-anesthetized rats were depressed by stimulation of autoreceptors with 100 µg/kg apomorphine (APO) (-)-AJ76 and (+)-UH 232 reversed APO effects with ED50's of 430±233 and 103±33 µg/kg, respectively. The greater potency for (+)-UH 232 as an autoreceptor antagonist contrasts with its weaker stimulant effects (Svensson et al., ibid) suggesting that, compared to (+)-AJ76, it is a less specific antagonist for autoreceptors.

In contrast, treatment with either antagonist elevated APO's ED50. (-)-AJ76 did not alter firing rates of 5-HT neurons in dorsal raphe. Using standard 2-DG protocol (Kolofoff et al., J. Neurochem. 28:897, 1977), 115 mg/kg (+)-AJ76 injected 1 min prior to 2-DG increased glucose metabolism in SNPC, VTA, globus pallidus, and n. accumbens, sites also stimulated by dopamine agonists. Locus coeruleus metabolism was also increased. Like dopamine postynaptic antagonist antipsychotics, (-)-AJ76 stimulated the lateral habenula. It is concluded that (+)-AJ76 is a selective DA autoreceptor antagonist.

378.14


NO-112 and NO-756 (+)-8-chloro-L-hydroxy-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-1H-3-benzazepin and (+)-8-chloro-L-hydroxy-1-methyl-5-(7-dihydrobenzofuranyl)-2,3,4,5-tetrahydro-1H-3-benzazepin are new selective D-1 receptor antagonists (see Andersen P.H. et al. this meeting). The drugs inhibited amphetamine-induced discriminative effects in very low doses (0.02 mg/kg) and also blocked methylphenidate or amphetamine-induced stereotyped behavior, conditioned avoidance responding and D-1 receptor mediated rotational behavior in the same low doses. These effects were obtained at lower D-1 receptor occupancy in vivo when compared with the occupation of D-2 receptors required for obtaining the same effects. When coupled with a large body of data implicating a potential role for D-1 receptors in controlling psychosis, the profile of NO-112 and NO-756 suggests that they may exert anti-psychotic action in the clinic.

379.1

DEVELOPMENT OF A THICK MEDIULLARY SLICE PREPARATION FOR THE STUDY OF RESPIRATORY RHYTHM GENERATION. B. Mclean and J. Remmers, Department of Medicine and Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The location of neurons critical to the generation and maintenance of respiratory rhythm is unknown. We have developed a preparation from the brainstem of the neonatal rat (1-3 days old) in which diffusion distances are considerably reduced but respiratory activity and chemosensitivity are maintained. The preparation consists of a thick slice of brainstem extending 1 mm rostral and 1 mm caudal to the obex. Respiratory activity is recorded from the rostral hypoglossal nerve roots. The preparation is maintained at 37°C, superfused with mock CSF equilibrated with 95% O2 and 5% CO2 and is viable for approximately 4 hours. The thick slice preparation demonstrates more rapid and pronounced frequency responses to alterations in super fusate PO2 than the "intact" in vitro brainstem-spinal cord preparation, suggesting better diffusible accessibility of O2 to the central chemoreceptor. Preliminary experiments indicate that ventral regions of the medulla contain neural elements adequate for rhythm generation and chemosensitivity. In addition, when the rostral aspect of the medulla is removed, the respiratory rhythm is decreased suggesting that this area contains a frequency promoting region. This preparation presents a potentially useful model for investigation of the neural substrate for respiratory rhythm generation.

Supported by grants MRC RMA-9719 and CPSID.

379.2

LOCALIZATION OF A MEDULLARY AREA SENSITIVE TO CHANGES IN CO2 IN IN VITRO BRAINSTEM-SPINAL CORD PREPARATION OF NEWBORN RATS. F. Issa* and J. Remmers. Dept of Medicine, Univ of Calgary, Calgary, Alberta, Canada T2N 4N1.

The exact location of the central chemoreceptor has not yet been determined. In the in vitro brainstem-spinal cord preparation of 1-3 day old rats was used to identify areas within the medulla sensitive to changes in CO2. The preparation was superfused with a mock CSF solution equilibrated with 3% CO2-95% O2 and kept at 38±1°C. A specially constructed compound glass micropipette with a tip diameter of 12-15 µm was used. The micropipette was filled with a mock CSF solution with a pH of 7.35. It was advanced in 50 µm steps into the ventral surface of the medulla using a stepping microdrive. At each depth, pressure ejection of 2-10 nl of CO2-enriched mock CSF solution was performed while the inspiratory activity of C2-C4 ventral rootlets was continuously monitored. Slowing of breathing from a control of 10-15 to 1-5 bursts/min was observed with injections made at depths of 0-200 µm in the area bound by hypoglossal nerve rootlets. An immediate and profound increase in breathing rate and amplitude of integrated C2-C4 neural activity occurred with injections in the area of 0.50-0.75 mm lateral to the midline, 0.5-0.8 mm rostral to the obex and at a depth of 0.25-0.35 mm from the ventral surface of the medulla. We conclude that neuronal elements in the medulla required to the obex, sense a change in PCO2 and augment breathing.

Supported by grants MRC RMA-9719 and AHPMR 8731.
INTERACTIONS BETWEEN THE A-CURRENT AND CALCIUM CURRENT IN BULBOSPINAL NEURONS IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS. M.S. Daxin, T.H. Morgan School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

The dorsal motor nucleus of the vagus in guinea pigs is located within the ventral part of the nucleus tractus solitarius (ventral NTS). Two classes of bulbospinal neurons, termed types I and II, have been identified within the ventral NTS. Both classes of cells express A-current (IA) and calcium current (IC). During depolarization, interactions between IC and IA will affect repetitive firing activity. In type I neurons, this interaction is manifest as a low frequency burst of spike activity while in type II neurons a long duration burst is seen. (Supported by NIH grants HL36050 and HL37146 (J.R.H.).)

CHARACTERISTICS OF POST-INHIBITORY REBOUND IN NEURONS WITHIN THE NUCLEUS AMBIGUUS OF ADULT GUINEA PIGS. S.M. Johnson* and P.A. Getting, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

We are interested in the intrinsic properties of neurons that participate in the formation of the respiratory motor pattern in mammals. The Nucleus Ambiguus (NA) contains a concentration of heterogeneous respiratory-related neurons including bulbospinal neurons, propriobulbar neurons, and vagal and glossopharyngeal motor neurons. Respiratory properties of these neurons were assessed by recording intracellularly (N = 150 cells) from transverse brain stem slices (350-400 microns thick, 0.5-1.5 mm caudally). Numerous serotonin immunoreactive boutons were observed in synaptic contact with phrenic motoneurons. These anatomical findings are indicative of a direct serotonergic innervation of the phrenic motor nucleus. (Supported by NIH grants HL33831 and NINCDS Training Grant 5-T32-NS07166-08.)

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SEROTONIN IMMUNOREACTIVE BOUTONS MAKE CLOSE CONTACTS WITH FELINE PHRENIC MOTONEURONS. J. Alipietz, P.M. Pillyay*, N.D. Voas* and D.de Castro*, ISPRN: R.Milne) Department of Physiology, University of Auckland, New Zealand.

There is a large body of evidence indicating that serotonergic mechanisms are involved in the central control of respiration. It has been suggested that this involvement occurs indirectly, through an action by serotonin-containing neurons on motoneurons. In the present study we have investigated the possibility of a direct input by serotonin neurons onto phrenic motor neurons.


GABA is a major inhibitory neurotransmitter which has a widespread distribution in the central nervous system. In the present report we describe its distribution in the phrenic motor nucleus of the cat both at the light and electron microscopic levels. Using a combination of immunocytochemical techniques on paraffin embedded material and electron microscope autoradiography, GABA was found to be localized in the synaptic terminals of phrenic motoneurons. GABA immunoreactivity was located as diffuse reaction product within terminals containing small, clear vesicles. In some synaptic contacts with neurons and glial processes the GABA immunoreactive terminals were unlabelled. The presence of GABA immunoreactive terminals that contact phrenic motoneurons provides morphological evidence that GABA is involved in synaptic circuitry that control the movement of the diaphragm. (Supported by NIH grant NS23861 (B.E.M.) and NIH grants HL36050 and HL7466 (J.R.H.).)

We have studied the effect of benzodiazepines on the inhibitory postsynaptic potentials (IPSs) and on the hyperpolarizing response to iontophoresed γ-aminobutyric acid (GABA) in bulbospinal motoneurons obtained from decerebrate, paralyzed cats. Diazepam (0.05-0.1 mg/kg i.v.) hyperpolarized the membrane in all inspiratory and postinspiratory neurons penetrated in the ventral respiratory group. Waves of IPSs occurring during "inactive" phase of the respiratory cycle substantially increased and the inspiratory IPSPs of each neuron. The effect during "active" phase include hyperpolarization associated with reduction of the input resistance, a decrease in the firing rate and shortening of the burst duration. Diazepam had no effect on the excitatory post synaptic potentials induced either by the vagus or the somatosensory stimulation, whereas it increased the stimulus-induced PSPs. Iontophoresis of flurazepam enhanced hyperpolarization induced by iontophoresed GABA. These results document that benzodiazepines potentiate specifically the phasic and tonic postsynaptic inhibitions in bulbar respiratory neurons, at least part of which are mediated by GABA.

379.11 SYSTEMIC ADMINISTRATION OF THE N-METHYL-D-ASPARTATE ACID (NMDA) RECEPTOR ANTAGONIST, MK-801 PRODUCES PRONOUNCED CHANGES IN CARDIOPULMONARY FUNCTION. T.F. Abraham*, R.A. Gills*, P. Hamosh*, and A. Mataierra da Silva*.(SPONS: T. Tizabi). Departments of Pharmacology, Physiology and Medicine, Georgetown University Medical School, Washington D.C. 20007.

Because of the potential role of CNS excitatory amino acids in the control of cardiovascular function, we evaluated the effects of MK-801 on several indices of cardiopulmonary activity in chloralose-anesthetized cats. I.V. doses of 0.03, 0.1, 0.3 and 1 mg/kg produced decreases in respiratory minute volume (Ve) of 4519, 145425, 285155 and 325298 ml/min, respectively. The decrease in the respiratory rate occurred due to both decreases in tidal volume (Vt) and respiratory rate (f). The decrease in Vt was due to prolongation of inspiratory duration (ti) as MK-801 (0.03, 0.1, 0.3 mg/kg) increased Vt by 0.850.1, 1.840.3 and 1.604.4 seconds respectively. The higher doses of MK-801 caused apneic breathing and apnea. Changes in arterial pressure (BP) occurred that were dose-related. An initial increase (within 1 min) followed by a later decrease in BP (after 5 min of drug administration) were observed. No significant changes in heart rate were noted. These results indicate that NMDA receptor blockade with MK-801 causes respiratory depression and significant changes in BP (supported by MH24222).


It has been reported that systemic administration of clonidine will protect rodents against the toxic effects of soman (GD) (Buccofigures, 1986). Since GD exerts respiratory effects at the level of the ventral surface of the medulla (VSM) (Gillis et al., 1987), we determined whether GD-induced respiratory changes could be counteracted by prior administration of clonidine applied at the VSM. GD (0.04 ug/side) applied bilaterally to the VSM of the chloralose-anesthetized cat decreased respiratory rate (f) by 9 + 2 breaths/min (from 15 + 1 breaths/min) and increased tidal volume (Vt) by 53 + 15 ml (from 32 ± 2 ml); respiratory minute volume remained unchanged. Clonidine (1 ug/side) applied to the VSM 5 min before GD counteracted both the GD-induced decrease in f and the increase in Vt. GD (0.4 ug/side) produced apnea in 5 of 9 animals tested due to a decrease in f. Animals pretreated with clonidine did not exhibit apnea (0/4) when GD (0.4 ug/side) was applied. These data suggest that the protective effect of clonidine against GD toxicity may occur because of an interaction of the 2 drugs at the VSM. (Supported by the US Army Med. & Development Command, Contract #DAMD17-86-06-034).

379.13 ROSTRAL VENTROLATERAL MEDULLA M: MUSCARINIC RECEPTOR INVOLVEMENT IN CENTRAL VENTILATORY CHEMOSENSITIVITY. E. Hatt*, J. Woods, A. Mega*, and W. Gorteks*, Department of Physiology, Dartmouth Medical School, Hanover, NH 03756. (SPONS: F. Mccann.)

Muscarinic agonist atropine (4.4 μm), the M1" receptor antagonist pirenzepine (10 μm), and the M2 antagonist AF-DX 116 (10 μm) were applied by cotton wicks to the rostral ventrolateral medulla (VLM) to test for muscarinic involvement of ventilatory chemosensory area in separate anesthetized, paralyzed, vagotomized, glicotecimized, and servo-ventilated cats with intact vagicrophic nerve activity used as a respiratory center output. Atropine or pirenzepine significantly decreased the O2 response slope 48.7% +/− 6.2 and 40.7% +/− 6.0 respectively, a significant reduction occurred in the maximal response value 26.3% +/− 8.1 and 19.2% +/− 3.2 respectively without significant effects on blood pressure. AF-DX 116 did not significantly affect on phrenic output or blood pressure. Atropine or pirenzepine had no significant effect on the phrenic response to carotid sinus nerve stimulation; these results suggest the involvement of muscarinic cholinergic receptors of the M1 subtype in the central CO2 chemoreceptor process accessible to the root of the rostral ventrolateral medulla. (Supported by NIH HL 82066.)

Pirenzepine and AF-DX 116 supplied generously by Boehringer Ingelheim, Inc.)
EXCITATORY AMINO ACIDS VII

380.1
NMDA RECEPTOR MEDIATES THE INITIAL SYNAPTIC RESPONSE IN SPINAL MOTONEURONS OF RAT EMBRYOS. L. Ziskind-Conhaim. Dept. Physiol., Univ. of Wisconsin, Madison, WI 53706. Formation of sensory motoneuron contacts was studied in isolated spinal cord of rat embryos at 15-21 days of gestation using intracellular recording and HRP labeling (Ziskind-Conhaim, L., Dev. Biol. 128:1988). At Days 15-16, when most afferents terminated at the intermediate gray matter, a few root fibers generated polysynaptic potentials. By Day 17, mono- and polysynaptic potentials were recorded in 60% of motoneurons, and HRP labeled afferents made synaptic contacts with motoneurons. In the vertebrate central nervous system, at least 2 receptor subtypes (NMDA and kainate/quisqualate receptors) mediate the excitatory response to L-glutamate. At the onset of sensory innervation, the amplitude of polysynaptic potentials increased with depolarizing currents injected intracellularly and with removal of extracellular Mg2+, a voltage-dependent blocker of the NMDA receptor. The prolonged potentiation induced by the removal of Mg2+ was blocked by NMDA antagonist. These manipulations did not change the amplitude of the monosynaptic responses. These results suggest that NMDA receptor mediates the initial polysynaptic response in the motoneurons of rat embryos. Conductance changes indicate that during embryonic development motoneuron sensitivity to NMDA and kainate was higher than to L-glutamate. Supported by NIH grant NS 23808 and by Spinal Cord Research Foundation grant NSR-4695-9.

380.2
ROLE OF EXCITATORY AMINO ACIDS (EAA) IN TRANSMISSION OF RESPIRATORY DRIVE TO PHRENIC MOTONEURONS I: KAINATE AND QUISQUALATE RECEPTORS. G. Liu, J.C. Smith, & J.L. Feldman. Systems Neuroscience Laboratory, Department of Neurobiology, UCLA, Los Angeles, CA 90024-1356. We previously demonstrated that excitatory amino acids are likely to play an important role in synaptic transmission of respiratory drive (Fed. Proc. 45:519, 1986). Mechanisms of actions of excitatory amino acids on phrenic motoneurons and AP4 receptors were studied by intracellular recordings from phrenic motoneurons in the in vitro neonatal rat brainstem-spinal cord preparation. We tested the effects of blocking EAA receptors on transmission of respiratory drive, 2 mM Kynurenic acid (KYN), an antagonist acting on all three subtypes of EAA receptors, was applied to the solution bathing the spinal cord (Fed. Proc. 45:519, 1986). We report the effects of drugs affecting kainate and quisqualate receptor subtypes on phrenic motoneuronal resting membrane potential, respiratory drive potential and membrane conductance in the in vitro spinal cord preparation (J. Neurosci. Meth. 2:321, 1987). To test the effects of blocking EAA receptors on transmission of respiratory drive, 2 mM Kynurenic acid (KYN), a non-NMDA receptor antagonist. These results suggested that non-NMDA receptors play an important role in neurotransmission of respiratory drive. Thus, we tested the effects of a potential non-NMDA receptor antagonist, 6-cyano-7-nitroquinazoline-2,3-dione (CNQX). Bath application of 20 µM CNQX depressed respiratory drive and decreased receptor-mediated conductance changes, resulting in abolition of respiratory-driven action potentials. Following washout, phrenic motoneuronal discharge completely recovered. These results suggest that non-NMDA receptors are important in the synaptic transmission of respiratory drive to phrenic motoneurons. This work was supported by NIH Grant NS 24742. J.C.S. is a Parker B. Francis Foundation Fellow.

380.3
ROLE OF EXCITATORY AMINO ACIDS IN TRANSMISSION OF RESPIRATORY DRIVE TO PHRENIC MOTONEURONS II: N-METHYL-D-ASPARTIC ACID (NMDA) AND 2-AMINO-4-PHOSPHONOBUTYRIC ACID-SENSITIVE (AP4) RECEPTORS. J.J. Feldman, G. Liu, & J.C. Smith. Systems Neuroscience Laboratory, Department of Neurobiology, UCLA, Los Angeles, CA 90024-1356. Excitatory amino acids are likely to play an important role in bulbospinal transmission of respiratory drive (Fed. Proc. 45:519, 1986). Mechanisms of actions of excitatory amino acids on phrenic motoneurons and AP4 receptors were studied by intracellular recordings from phrenic motoneurons in the in vitro neonatal rat brainstem-spinal cord preparation. Addition of 200 µM N-methylaspartic acid (AP4), a specific NMDA antagonist, to the bathing medium surrounding the spinal cord, produced a moderate reduction in resting potential. Increasing AP4 concentrations (1 mM) did not abolish phrenic motoneuronal firing. Given the relatively high concentration of AP4 in the bathing medium, the effects may be due to non-NMDA receptors. AP4 at low concentrations blocked action potentials and depressed driving potential. AP4 blocked synaptic potentials and synaptic potentials completely blocked phrenic motoneuronal activity within 1-2 min. The prolonged potentiation induced by the removal of Mg2+, a voltage-dependent blocker of the NMDA receptor, was repleted by hyperventilating animals and infusing glucose (24% increase in whole muscle glycogen content after 8h, P<0.05). Then animals were spontaneously breathing at normal oxygen levels for 1.5 h. Phrenic motoneuron discharge completely recovered. These results suggest that non-NMDA receptors plays an important role in neurotransmission of respiratory drive. Thus, we tested the effects of blocking EAA receptors on transmission of respiratory drive, 2 mM Kynurenic acid (KYN), an antagonist acting on all three subtypes of EAA receptors, was applied to the solution bathing the spinal cord (Fed. Proc. 45:519, 1986). We report the effects of drugs affecting kainate and quisqualate receptor subtypes on phrenic motoneuronal resting membrane potential, respiratory drive potential and membrane conductance in the in vitro spinal cord preparation (J. Neurosci. Meth. 2:321, 1987). To test the effects of blocking EAA receptors on transmission of respiratory drive, 2 mM Kynurenic acid (KYN), a non-NMDA receptor antagonist. These results suggested that non-NMDA receptors play an important role in neurotransmission of respiratory drive. Thus, we tested the effects of a potential non-NMDA receptor antagonist, 6-cyano-7-nitroquinazoline-2,3-dione (CNQX). Bath application of 20 µM CNQX depressed respiratory drive and decreased receptor-mediated conductance changes, resulting in abolition of respiratory-driven action potentials. Following washout, phrenic motoneuronal discharge completely recovered. These results suggest that non-NMDA receptors are important in the synaptic transmission of respiratory drive to phrenic motoneurons. This work was supported by NIH Grant NS 24742. J.C.S. is a Parker B. Francis Foundation Fellow.

380.4
THE NMDA RECEPTOR: CENTRAL ROLE IN MEDIATING PAIN INHIBITION IN RAT PERIAQUEDUCTAL GRAY. Y. F. Jacquemet. Behavioral Neuropharmacology Lab., Nathan Eline Institute, Orangeburg, NY 10962. The role of the periaqueductal gray (PAG) in the mediation of opiate analgesia is well established (Jacquet & Lajtha, Science, 1974). An injection of morphine in the PAG resulted in a naloxone-reversible analgesia, indicating an opiate receptor mediated action. The present study investigated what role, if any, the excitatory amino acids (EAA) may also play in pain inhibition at this CNS site. An injection of the EAA analogue, N-methyl-D-aspartate (NMDA) (10 nmol) in the rat PAG resulted in potent analgesia in 2 analgesia tests (Tail flick & Nociceptor Pinch). A prior injection of the NMDA antagonist, (+-2-amino-7-phosphonooctanoate (D-AP7) (300 nmol) antagonized this action, indicating a receptor-mediated action. D-AP7 also completely blocked morphine analgesia (NMDA) given with morphine (25 nmol) potentiated morphine analgesia, while the opiate antagonist, naloxone (5 nmol), only partially reversed this analgesia action. These results are consistent with the view that opiate-mediated analgesia in the PAG may be due to a disinhibitory action on an excitatory descending pain inhibitory pathway (Basham & Fields, Ann Rev Neurosci., 1984). The present findings delineate for the first time a functional role for the NMDA receptor in the control of pain in the mammalian central nervous system.
380.5
ALTERATION OF EXTRACELLULAR GLUTAMATE AND GLUTAMINE LEVELS DUE TO VIBRISSEAL STIMULATION IN THE VENTROPOSTERIOR MEDIAL (VPM) DURING VIBRISSEAL STIMULATION AND FOLLOWING ABLATION OF THESomatosensory Cortex. D.T. Ross. Department of Clinical Neurosciences, Brown University and Department of Neurosurgery, Rhode Island Hospital, Providence, RI 02902.

An excitatory amino acid agonist has been implicated as the neurotransmitter at lamellar synapses in the thalamic ventrobasal complex on the basis of immunohistochemical, physiological, and behavioral studies. In order to measure the release of extracellular amino acids during physiological stimulation in vivo microdialysis was performed on the surface of the VPM.

A microdialysis probe with a 2 mm long tip was stereotaxically implanted in the VPM at a 45° angle along the long axis of the nucleus. Dialyse samples were collected for 20 minutes at baseline and 20 minutes following the stimulation of the VPM. Baseline samples were collected during normal spontaneous activity, and stimulation samples were collected at the time of vibrisseal stimulation.

The extracellular concentration of glutamate and glutamine during bilateral stimulation of the VPM was significantly greater than baseline levels (p<0.05). The increase in extracellular levels of glutamate and glutamine was greatest during stimulation of the VPM and the increase in the level of glutamine was greatest. Baseline levels of aspartate in the VPM were very low and vibrisseal stimulation did not produce a detectable increase. The extracellular concentration of glutamate and glutamine collected during ablation of the somatosensory cortex and for the subsequent 20 minutes were significantly lower than baseline levels (p<0.05). These results indicate that the decrease in spontaneous activity recorded in the VPM immediately after cortical ablation is associated with a decrease in extracellular amino acids in the VPM during cortical stimulation and at longer times following cortical ablation.

380.7
FAST EPSPS EVOCKED IN THE GOLDISH MAUTHNER CELL BY SENSORY AFERENTS ARE DUE TO NMDA RECEPTOR ACTIVATION. L. G. Wood** and D. S. Faber (SPON: J. Whitney). Dept. of Physiology, S.U.N.Y. at Buffalo, Buffalo, NY, 14214.

Saccular afferents terminate as mixed (electrotonic and chemical) synapses on the inner cell layer of the Mauthner cell. Depolarization produced by their impulses have time constants of decay in the range of 1-2 ms. To determine whether the transmitter at these synapses is an excitatory amino acid, we have pre-injected the glutamate antagonist, 2,3-diaminoglycine (d-lyg), and 2-amino-4-phenylphosphonic acid (APA) onto the lateral dendrite while recording, from that dendrite, epsps evoked by the saccular afferents. The effects of these compounds on EPSPs arising from both single- and pooled-pulse stimulation were examined. Neither drug altered resting membrane potential or antidromic spike height. d-lyg completely abolished both the unconditioned epsps and the facilitated epsps, whereas APA reduced each by at least 80-90%, indicating that the receptors at these synapses are predominantly of the NMDA subtype. For both compounds, the second, pooled facilitated epsps was more sensitive to the antagonist than was the first.

We conclude that this fast epsp is primarily due to the activation of NMDA receptors and that the pooled-pulse facilitated of this epsp, seen with stimulation of a population of afferents may involve a postsynaptic mechanism, in addition to the presynaptic one described previously for single fibers (I. Whitney). J. Neurosci. (d): 1313-1325 (Supported by NIH NS1848 and 15335).

380.8
A-DELTA AND C AFFERENT FIBER-EVOKED SYNAPTIC RESPONSES OF RAT SUBSTANTIA GELATINOSA NEURONS IN VITRO. M. Yasminov and T.M. Hughes. Center for Neurobiology & Health Hughes Medical Institute, Columbia University, New York, NY 10032.

Primary afferent-fiber-evoked synaptic responses of substantia gelatinosa (s.g.) neurons have been studied by intracellular recording in a transverse slice preparation of adult rat spinal cord that retains an attached dorsal root. Neurons in s.g. exhibited A-delta and/or C fiber mediated monosynaptic fast epsps in response to dorsal root stimulation. A and A-delta fiber-mediated epsps had similar time courses and amplitudes. To study C fiber input selectively we have used low concentrations (0.05 µM) of TTX that preferentially block the activation of A-delta fibers without substantially affecting C fiber evoked epsps. Higher concentrations of TTX (0.5 µM) blocked C fiber responses.

Both A-delta and C fiber evoked epsps were increased in amplitude with membrane hyperpolarization and decreased with membrane depolarization and reversed in polarity at membrane potentials between -10 and 0 mV. In the presence of TTX (0.5 µM), l-glutamate (0.5 - 5 µM) produced a membrane depolarization in 40-50% of s.g. neurons. The l-glutamate-induced depolarization reversed in polarity at the same membrane potential as that of afferent-evoked epsps. The amino acid antagonist kynurenic acid (1 mM) depressed the amplitude of A-delta and C fiber mediated epsps and l-glutamate response in some but not all s.g. neurons.

These results indicate that some but perhaps not all A-delta and C fiber afferents release amino acids as fast excitatory transmitters at afferent synapses with s.g. neurons.

380.10

Swallowing is a motor activity generated by a central pattern generator including leading neurons located in the nucleus tractus solitarius (NTS). The sequential motor pattern characteristic of swallowing, linked with the inhibition of respiration, is routinely elicited in anesthetized rats by stimulation of the laryngeal afferents. The same motor pattern was also selectively generated by glutamate or excitatory amino acids (EAA) agonists (quisqualate, N-methyl-D-aspartate (NMDA)) applied directly into the swallowing region of the NTS (pressure injections: 5 nL, 0.05-0.5 mM). Injections of a depolarizing agent (KCl, 0.02-0.2 M) were ineffective. When initiated by stimulation of laryngeal afferents was blocked by microinjections, into the active sites, of EAA antagonists (glutamate diethylster, -glutamylaminomethyl sulfonate, 2-amino-5-phosphonovaleric acid; 0.5-5 mM). These results suggest that EAA might be transmitters involved in the initiation of swallowing by laryngeal afferents. Retrograde labeling of nodose cell bodies after injection of 5-9-aspasartate (100 µ Ci) in the swallowing region of the NTS suggest that certain vagal afferent fibers use EAA as transmitters.

380.9

We have characterized several synaptic pathways onto pyramidal neurons which can be selectively activated in rat piriform cortex slices. The pathway activated by stimulation of the lateral olfactory tract (LOI) is blocked by amino-phophoryl acetic acid (APA) and L-glutamate agonists, but not other excitatory amino acid antagonists. This pathway shows paired pulse (PPF) and long term potentiation (LTP), and A- and C afferents terminate on distal apical dendrites. Stimulation deep in the slice, which activates association fibers, elicits two distinct excitatory responses on pyramidal neurons. Both components are blocked by kynurenic acid, but not APA or the N-methyl-D-aspartic acid (NMDA) antagonist, D -cyclizine. Neither component shows LTP, and only the faster shows PPF. Both components reverse deeper in the slice than the L0T inputs, the slower in the proximal apical dendrites while the faster appears to terminate on basal dendrites. GABAergic inhibition of C afferent mediated responses is elicited by subthreshold stimulation, and terminates near the cell bodies. Acetylcholine receptors are located exclusively on pyramidal neurons, and close voltage dependent N channels.
The effects of the proposed competitive N-methyl-D-aspartate (NMDA) antagonists share certain behavioral effects with the non-competitive NMDA antagonists. However, the selectivity of these drugs to produce only PCP-like effects has not been well-studied. We investigated whether the competitive NMDA antagonist 3-((+/-) -2-carboxy-piperazin-4-ylpropyl-1-phosphonic acid (CPP) and pentobarbital (PB) in rats trained to discriminate 1.25 mg/kg i.p. phencyclidine (PCP) from saline. Both CPP (30 mg/kg i.p.) and NPC 12626 (100 mg/kg i.p.) dose-dependently reduced response rates but failed to completely substitute for PCP. Selective effects were observed with PB. For all three drugs, PCP-lever responding was usually associated with increased locomotor activity, and response rate decreases often occurred without evidence of generalization. These results suggest that there is not a complete overlap in the discriminative effects of PCP and NPC 12626, CPP and PB. Furthermore, behavioral effects other than PCP-like effects are produced by these drugs, and the effects of competitive NMDA antagonists are no more PCP-like than are the effects of PB.

(Partially supported by NIDA Grant DA-01442.)

Kainic acid (KA), α-amino-3-hydroxy-5-methylisoxazole-4-proponate (AMPA), and N-methyl-D-aspartic acid (NMDA) stimulate locomotor activity immediately after injection into the substantia innominata/lateral preoptic area (SI/LP). P. E. Shreve* and N. J. Uretsky, The Ohio State University College of Pharmacy, Columbus, Ohio 43210.

The SI/LP is a region of the ventral pallidum which appears to be a critical neural substrate for LMA initiated in the nucleus accumbens (NA) through a GABAergic projection from the NA. Recent evidence suggests that the SI/LP also receives glutamatergic innervation. The purpose of these experiments was to determine the effects of excitatory amino acids (EAA) on LMA after direct injection into the SI/LP. Various doses of KA, AMPA, and NPC were injected into the SI/LP and LMA was recorded. KA (15-60 mg), AMPA (0.1-1 µg), and NMDA (1-2.5 µg) produced dose-dependent increases in LMA which was similar to that observed in the NA. α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) has been shown to be a selective quisqualic acid (QA) receptor antagonist in the NA. In the present study, AMPA (1 µg) produced a selective inhibition (32%) of AMPA (0.5 µg) while having no significant effect on KA (30 mg) or NMDA (2.5 µg). These results suggest that QA receptors mediate the LMA produced by AMPA and that activation of SI/LP by glutamate may play a role in modulating goal-oriented behavior initiated in the NA. Supported by NS 22582.

EFFECTS OF NMDA, L-GLUTAMIC ACID AND L-ASPARTIC ACID ON AUDITORY AM) 380.14

The excitatory amino acid, N-methyl-D-aspartate (NMDA), L-glutamic acid (GLU) and L-aspartic acid (ASP) have been shown in our laboratory to exhibit conproconvulsive activity in the mouse electroconvulsive seizure model. The present study was designed to evaluate the actions of these three compounds, at two effective and one ineffective dose in kindled animals. We examined the effects of intraventricular administration of kynurenic acid on locomotor behavior in rats. A digoxin assay was used to quantify the levels of kynurenic acid in the SI/LP. The effects of intraventricular administration of kynurenic acid on locomotor activity in the SI/LP were examined in a standard kindling procedure. Movement data were collected on the first day and every third day thereafter. A repeated measure design was used to analyze activity and vertical activity. The results indicated that both dose and saline were significantly different from each other. The effects of kynurenic acid on locomotor activity and vertical (rear) activity were not the same. Further analyses revealed different activity patterns within test sessions for various doses of kynurenic acid.

Supported by NSERC.
EXCITATORY AMINO ACIDS VII

Heckendorn* & J.G. Dingwall*. Friedrich Miescher Institute, Central Research Labs., & Research & Development Dept., Pharmaceuticals

methyl-D-aspartate (NMDA) receptor antagonists, we investigated a series of binding sites in rat brain synaptic membranes and as antagonists of selected on the basis of their activity at NMDA-sensitive L-3H-glutamate (kainate). Sub-micromolar concentrations blocked NMDA-, but not rat hippocampal slice potential candidates for the treatment of disorders such as epilepsy and 37849 and its ethyl ester CGP 39551 (which was weaker in assays for 17 other receptor types (including quisqualate and inactive in assays for 17 other receptor types (including quisqualate and +KCNQ1), and may have difficulty recalling what he had learned when tested 24 hrs later. Supported by Research Scientist Award MH 38894 (JWO), AG 05681 and MH 14677.

Several experiments were conducted to study the effects of MK-801 on learning and memory in rats. In one study, rats were trained to an task involving the reversal of a position habit. The protocol consisted of 3-day sessions, with 15 trials per session. Rats in the experimental group were administered MK-801 1 h prior to treatment. First, MK-801 (10.0 mg/kg) was administered to rats prior to increasing doses of N-MDA (5.10, and 20 ug/site). Next, two doses of MK-801 (1.0 and 0.1 mg/kg) were administered prior to one dose of N-MDA (10 ug/site). In both experiments, motor activity was measured 1, 2, and 3 wk after surgery. Animals receiving N-MDA were trained for acquisition of a Morris water maze task 4 wk post-surgery. 10 mg/kg MK-801 completely blocked increased in motor activity produced by 10 and 20 ug/site N-MDA while both 1.0 and 1.0 mg/kg MK-801 blocked 10 ug/site N-MDA-induced hyperactivity. In addition, 10.0 and 1.0 mg/kg MK-801 completely attenuated water maze acquisition deficits produced by N-MDA. These results were confirmed in chronic cats given MK-801 50 ug/kg MK-801 (0.1 mg/kg) given a 1-trial test to see if they would respond correctly, i.e., go to the side opposed to the one in which they were reinforced on the previous day. Again, rats were required to make 9 out of 10 consecutive responses to the reinforced side. On day 3, they were given a 1-trial test. If they responded correctly, they were allowed to go to the side reinforced on day 2. The same experiment was repeated once a week for 3 consecutive experimental and control groups. The number of saline control rats which responded correctly on the 1-trial test was significantly greater than levels expected by chance for each of the three weekly experiments whereas MK-801 rats consistently failed to perform above chance levels. Performance deficits on day 3 could not be attributed to motor impairment since the dose of MK-801 is well below equipotent concentrations of the non-NMDA and NMDA antagonists used in these studies. These results suggest that NMDA receptors may play a role in the effects of MK-801 on memory. Supported by grants NS2346 and MH43362.

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A10 dopamine neurons in the A10 region? Glutamatic acid, aspartic acid, kainic acid or N-methyl-D-aspartate (NMDA) were injected into the A10 region of rats. Changes in motor activity and DA metabolism measures of glucose and kainate were performed. A dose-related increase in horizontal photocell counts with a minimum effective dose (MED) of 100 and 1 mlones, resp. The dose response curves for N-MDA and MED were biphasic. An increase in activity was seen at high doses of NMDA and aspartate (1 and 100 mlones), while a decrease occurred after lower doses (0.01 to 0.1 mg/ml). When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection. When a behavioral stimulant dose of kainate and glucose was injected into the A10 region, a significant increase in DA metabolism was produced that lasted for 3 to 4 min after the injection.
381.3

LOSS OF [3H]GLUTAMATE BINDING AFTER ORBITAL EMBOLIZATION J. McLulloch & D.P. Chain.* (SPON: G. Fink), Wellcome Surgical Institute, University of Glasgow, Glasgow G61 1QH.

Glutamate plays a major role within the rat visual system. Using a fully quantitative autoradiographic technique, we have examined the alterations in [3H]glutamate binding sites which accompany functional disturbances after lesions of this sensory pathway. Black-hooded Long Evans rats were unilaterally enucleated under 2% halothane anaesthesia and twenty-four hours later received a single glucose washout calculated for 45 min. at 4°C with 200mM [3H]-glutamate, non-specific binding being determined in the presence of 1µM unlabelled glutamate.

Orbital embolization produced a significant reduction in glucose use in the visually deprived superior colliculus, lateral geniculate nucleus and visual cortex. These functional disturbances were accompanied by a significant reduction in [3H]glutamate binding in both superficial and deep layers of visual cortex.

This rapid response to altered neuronal activity suggests an important functional role for this site in cortical excitatory neurotransmission. This response will be discussed in relation to specific receptor subtypes and binding conditions.

381.5

EFFECTS OF A SERIES OF 4-(PHOSPHONOALKYL)Piperidine-2-carboxylic Acid Derivatives and Mice as Antagonists of NMDA INDUCED CHEMICAL SEIZURES. J. D. Leander* AND J. W. Fischberg, Surgical Institute, University of Glasgow, Glasgow G61 1QH.

We recently prepared a series of 4-phosphonoalkylpiperidine-2-carboxylic acids (I-VII) n = 1, 2, 3; 1V, n = 2; IV, n = 3; IV, n = 4) as antagonists of NMDA-induced seizures in mice. In order to determine the extent of antagonistic activity, we examined these compounds in a variety of assays: production of phenoclyide (PCP)-like catalepsy and antagonism of NMDA-induced suppression of respiration in mice, protection from maximal electroshock (MES) induced convulsions in mice; protection from NMDA- and 3-mecaptopropiophenopic acid (3-MPA)-induced lethality in mice; and antagonism of NMDA binding sites which accompany functional disturbances were accompanied by a significant reduction in [3H]glutamate binding in both superficial and deep layers of visual cortex.

Compounds I and III produced PCP-like catalepsy (both 2.5 mg/kg, i.p., slow onset and long duration of action) and antagonized NMDA-induced suppression of respiration in mice (both 10 and 40 mg/kg). At doses that afford protection in the above assays, the mice were impaired on the horizontal screen. Compounds II and IV (at doses up to 160 mg/kg, i.p.) were inactive in mice.

The pattern of activity observed for this series parallels that observed for the acyclic series of ω-phosphono-α-amino acids, where AP5 and AP7 possessed NMDA antagonist activity while AP6 and AP8 were inactive. Reduction of the pipecolic ring led to enhanced potency relative to the analogous analogues.

381.6

ALTERATIONS IN RAT BRAIN METABOLIC ACTIVITY BY VARIOUS NON-COMPEITIVE NMDA ANTAGONISTS. M.H. Ennis*, J. Monne*, K. Rice* and A. Pert (SPON: L. Hsu). BPS, NMD, and IC, NIND.

The purpose of this study was to compare the effects of three non-competitive NMDA antagonists on the metabolic activity of rat brain. Rats were injected systemically with either phencyclidine (PCP), (-) SKF 10,047, MK-801 or saline. Thirty minutes later, all animals were injected with [3H]-glutamate. Standard procedures of visualizing 2-DG were employed.

Significant increases in metabolic activity were found in the anterior and posterior cingulate cortex, anterior, ventral, ventromedial and posterior thalamic nuclei, dorsal and ventral hippocampus, and substantia nigra (SN) following MK-801. (-) SKF 10,047, MK-801 and saline. Thirty minutes later, all animals were injected with [3H]-glutamate (2-DG). Standard procedures of visualizing 2-DG were employed.

Significant increases in metabolic activity were found in the anterior and posterior cingulate cortex, anterior, ventral, ventromedial and posterior thalamic nuclei, dorsal and ventral hippocampus, and substantia nigra (SN) following MK-801. (-) SKF 10,047. MK-801 enhanced metabolic activity in the anterior and posterior cingulate and the substantia nigra (SN) as well as the caudate nucleus. Unlike MK-801, however, PCP decreased metabolic activity in the thalamic nuclei. In general, both enantiomers of SKF 10,047 reduced the metabolic activity of a variety of structures, including the hippocampus, the major thalamic nuclei and the substantia nigra. It is clear that these non-competitive NMDA antagonists have vastly different consequences on brain metabolic activity.

381.7

CENTRALLY ADMINISTERED QUINOLIC ACID INCREASES SENSITIVITY TO DIETHYLBARBITURIC ACID IN LS AND SS MICE. C. C. Duncan and J. A. Ruth. University of Colorado School of Pharmacy, Box 297, Boulder, CO, 80309.

A gas-chromatographic assay for the determination of brain diethylbarbituric acid (DB) concentrations has been developed and used to study the role of glutamate neurotransmission in the modulation of the CNS depressant effects of this water soluble barbiturate in long-sleep (LS) and short-sleep (SS) mice. LS and SS mice are lines genetically selected for their differential response to acute ethanol as measured by duration of loss of righting response. We have previously demonstrated a differential involvement of the glutamate receptors in this response. The current study was designed to further investigate the role of NMDA receptors in the response to ethanol. Halothane anaesthetized mice were injected with [3H]-glutamate (14C)-2-deoxyglucose technique. (14C)-2-deoxyglucose-6-phosphate was eluted from sections purchased for receptor autoradiography and these were incubated for 45 min. at 4°C with 200mM [3H]-glutamate, non-specific binding being determined in the presence of 1µM unlabelled glutamate.

Orbital embolization produced a significant reduction in glucose use in the visually deprived superior colliculus, lateral geniculate nucleus and visual cortex. These functional disturbances were accompanied by a significant reduction in [3H]glutamate binding in both superficial and deep layers of visual cortex.

This rapid response to altered neuronal activity suggests an important functional role for this site in cortical excitatory neurotransmission. This response will be discussed in relation to specific receptor subtypes and binding conditions.

Compounds I and III produced PCP-like catalepsy (both 2.5 mg/kg, i.p., slow onset and long duration of action) and antagonized NMDA-induced suppression of respiration in mice (both 10 and 40 mg/kg). At doses that afford protection in the above assays, the mice were impaired on the horizontal screen. Compounds II and IV (at doses up to 160 mg/kg, i.p.) were inactive in mice.

The pattern of activity observed for this series parallels that observed for the acyclic series of ω-phosphono-α-amino acids, where AP5 and AP7 possessed NMDA antagonist activity while AP6 and AP8 were inactive. Reduction of the pipecolic ring led to enhanced potency relative to the analogous analogues.

381.8

NMDA RECEPTORS ARE INVOLVED IN THE ACUTE RESPONSE TO ETHANOL IN LS AND SS MICE. W.R. Wilson and J.A. Ruth. University of Colorado School of Pharmacy, Box 297, Boulder, CO, 80309.

LS and SS are lines of mice that have been genetically selected for their differential response to acute ethanol as measured by duration of loss of righting response. We have shown that the NMDA antagonist 2-amino-5-phosphonovaleric acid (APV) given by intravenous injection increased CNS sensitivity to ethanol 55% in LS and 45% in SS mice. NMDA had a small effect in LS mice but was without effect in SS mice. The current study was designed to further investigate the role of NMDA receptors in the response to ethanol. Halothane anaesthetized mice were injected with [3H]-glutamate (14C)-2-deoxyglucose technique. (14C)-2-deoxyglucose-6-phosphate was eluted from sections purchased for receptor autoradiography and these were incubated for 45 min. at 4°C with 200mM [3H]-glutamate, non-specific binding being determined in the presence of 1µM unlabelled glutamate.

Mice were injected IP with 15 mg/kg DB. DB brain concentrations were determined at loss of righting response. LS and SS differed significantly in central sensitivity to DB (77.0±7.5 µg/g brain in LS and 120.5±4.9 µg/g brain in SS). Time course studies showed that these differences were not due to differences in DB clearance rates. These data provide direct evidence for the differential sensitivity of LS and SS mice to the depressant effects of DB. The role of glutamate modulation of DB effects was studied by ICV administration of the NMDA antagonist, D-2-amino-5-phosphonovaleric acid (5-APV). 5-APV had no effect on brain sensitivity to DB as reflected by a 45% decrease in brain DB concentration which induced loss of righting. These data support a role of glutamate neurotransmission in the modulation of the central-depressant effects of water soluble barbiturates. Supported by USPHS grant AA-03527.

This study supports the hypothesis that NMDA receptors are involved in the acute response to ethanol in LS and SS mice and may be part of the biochemical profile that is involved in the differential response to ethanol in LS and SS mice. This work was supported by USPHS grant AA-03527.
EXCITATORY AMINO ACIDS VIII

THURSDAY AM

381.9 THYROTROPIN RELEASING HORMONE ENHANCES EXCITATORY SYNAPTIC TRANSMISSION IN VITRO IN PREGNANT TURTLE HIPPOCAMPUS. G. Larson-Prior and N. T. Slater. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611. U.S.A.

In pregnant turtle hippocampal neurones, the thyrotropin-releasing hormone (TRH) mediated neurone excitability was increased by a direct effect on the excitatory postsynaptic currents (EPSCs) of motoneurones. Pharmacological studies were performed on a total of 11 cells holding potentials of -80 to -50 mV, both before and after the addition of TRH (50 µM). No sustained inward or outward current was produced by TRH, but in some cases there was an increase in the frequency of spontaneous synapses. In 8 of the cells tested, TRH caused a significant (P < 0.002) increase in the duration of the EPSCs over the entire range of holding potentials used. The reversal potential of the EPSC was estimated, by extrapolation of the 1/e curve, to be approximately -5 mV and was unchanged by TRH. The I/V curves were nonlinear, particularly over the range -60 to -80 mV, suggesting a voltage dependence of the channels involved in the activation of the EPSC, reminiscent of those channels activated by exogenous glutamate in these neurones.

Our previous work has shown that the membrane conductance of motoneurones and their sensitivity to exogenous glutamate are unchanged by TRH. Together, these results provide evidence for a presynaptic site of action of TRH. This work is supported by the Wellcome Trust.

381.11 THE ROLE OF NMDA RECEPTOR MEDIATED CURRENTS IN THE MANIFESTATION OF LTP IN CORTICAL NEURONES. A. Artola and W. Singer. Max Planck Institute for Brain Research, D-6000 Frankfurt/M, F.R.G.

In cortical cells in rat visual cortex slices, reduction of GABAergic inhibition increases NMDA receptor mediated currents and facilitates long term potentiation (LTP) after tetanic stimulation (TE). By blocking synaptic responses we demonstrate that NMDA conductances are necessary for the induction and manifestation of LTP. In 8 of the cells tested, LTP caused a significant (P < 0.001) increase in the amplitude of the EPSCs over the poststimulus. At 0.1 to 0.2 µM, non-NMDA dependent, PSP component (e-PSP, 8-11 ms after TE) underwent LTP, with large ratio changes (91.3 ± 38.1%, n=11) both e- and 1-PSP showed LTP, which amounted to 58.9 ± 11.6% (n=8) only the 1-PSP was blocked by APV 20 min post-stimulus. Although LTP effects on direct NMDA actions were inconsistent, this study produced a 40% increase in NMDA enhancement of Quis excitation. No effects on kainate excitation were noted. In contrast, P depressor excitation responses of Pj cells to both Quis (33/36 cells) and kainate (101/11 cells) by 30-70%. Surprisingly, P also potentiated the modulatory effects of NMDA on Quis responses. This latter effect may moderate P effects on Glu physiology, as concurrent application of APV increased the magnitude of P suppression of Quis excitation by 10-20%, and prevented recovery to control levels of Quis excitation normally seen by 30 min post-P. Effects of the receptor antagonist were found to be predominantly mediated at the GABA(A) subtype, as i.) No effects of P on Bic inhibitions were observed and ii.) A dose of bicuculline resulting in a 40% reduction in Glu enhancement prevented P modulation of GABA responses. Furthermore, P modulation of LTP activation was determined to be independent of the GABA(A) receptor, as bicuculline application did not prevent P suppression of Quis excitation. (Supported by NS58698.)

381.12 EFFECTS OF NEW KAINATE RECEPTOR ANTAGONISTS ON MUDPUPPY RETINAL NEURON ELECTROPHYSIOLOGY. L.C. Stillwell and T.E. Tamarin. Dept. of Pharmacology, University of Washington, St. Louis, MO 63110. (spon. A.I. Cohen)

We have analyzed the action of a new putative kainate (KA) receptor antagonist using whole cell recording techniques in the intact mudpuppy retina (N. picturatus). The quinzine derivatives DNQX and CNQX inhibited the KA and quisqualate (QQ)-induced increases in the amplitude of the EPSCs over the poststimulus. These antagonists were both applied in ion exchange columns (1 µM in 400 µM NaCl, pH: 8.4), NMDA (50 µM in 400 µM NaCl, pH: 7.4), and MK-801 (50 µM in 200 µM NaCl, pH: 6.4). The central barrel was used for unitary recordings. The central antagonist was tested against the excitatory amino acid receptor agonists: quisqualate (1 mM in NaCl 400 mM, pH: 8), kainate and NMDA, as well as to specific NMDA antagonists such as APV, were tested before and after i.v. application of E2 (100 ng/kg) or P (50 µg). In addition to a direct cell response, continuous application of these antagonists produced marked increases (up to 400%) in Quis excitation (2230 cells). Differential effects of P on GABA(A)/GABA(B) receptor-mediated currents were also observed and balanced baclofen and bicuculline. The KA-enhancing actions of E2 appear to have two components: a fast (5-10 min) exerted at the Quis receptor and a later component involving NMDA receptor activation: E2 augmented Quis excitation by 100% in all cases (P < 0.02) only blocked by APV 20 min post-stimulus. Although E2 effects on direct NMDA actions were inconsistent, this study produced a 40% increase in NMDA enhancement of Quis excitation. No effects on kainate excitation were noted. In contrast, P depressor excitation responses of Pj cells to both Quis (33/36 cells) and kainate (101/11 cells) by 30-70%. Surprisingly, P also potentiated the modulatory effects of NMDA on Quis responses. This latter effect may moderate P effects on Glu physiology, as concurrent application of APV increased the magnitude of P suppression of Quis excitation by 10-20%, and prevented recovery to control levels of Quis excitation normally seen by 30 min post-P. Effects of the receptor antagonist were found to be predominantly mediated at the GABA(A) subtype, as i.) No effects of P on Bic inhibitions were observed and ii.) A dose of bicuculline resulting in a 40% reduction in Glu enhancement prevented P modulation of GABA responses. Furthermore, P modulation of LTP activation was determined to be independent of the GABA(A) receptor, as bicuculline application did not prevent P suppression of Quis excitation. (Supported by NS58698.)

381.13 BARIUM REVEALS NMDA RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION IN TURTLE HIPPOCAMPUS. Linda J. Larson-Prior and N. T. Slater. Dept. of Physiology, Northwestern University Medical School, 333 E. Chicago Avenue, Chicago, IL 60611. U.S.A.

In turtle hippocampal neurones, the barium (Ba2+) antagonist reduces convulsant-induced epileptiform discharges in turtle hippocampus (ventromedial cortex), but in the absence of convulsant drugs information transfer of YMCA pyramidal neurons evokes a biphasic EPSP which marks underlying excitatory transmission (1). Direct evidence for NMDA receptor-mediated transmission in this structure has been demonstrated by the bath application of 500 µM BaCl2 or permeation of cells with cesium-filled electrodes, both of which block NMDA- and K+-mediated IPSPs and reveal a late, slow EPSP (Fig. 1). This EPSP was reversibly blocked by the NMDA receptor antagonists D-AP5 (50-100 µM) and ketamine (250 µM).

CONTROL

D-AP5 (500 µM) + BaCl2 (500 µM)

D-AP5 (100 µM)

The slow time-course of the NMDA receptor-mediated EPSP was revealed by subtraction of responses before and after the addition of D-AP5 or ketamine. I-V plots of this potential did not display the expected voltage-dependent rectification in the presence of external magnesium. Voltage-clamp of neurones in barium or impaled with cesium-filled electrodes revealed a late, slow inward current which was reversibly blocked by D-AP5. These results demonstrate the existence of NMDA receptor-mediated excitatory transmission in turtle hippocampus that likely contributes to the high amplitude epileptiform discharges.


Behavioral and electrophysiological studies have shown that MK-801, an agonist of the PCP receptor, blocks specifically the excitatory and proconvulsant actions of N-methyl-D-aspartate (NMDA).

Male Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg, i.v.) and were microinjected with 1 µl of the drug. All experiments were used for extracellular recording and microiontophoresis. The central barrel was used for unitary recording and the side barrel for low voltage recordings: kainate (1 nM in NaCl 400 mM, pH: 8), quisqualate (1.5 mM in 400 nM NaCl, pH: 8), NMDA (50 mM in 400 nM NaCl, pH: 8), and MK-801 (50 µM in 200 mM NaCl, pH: 4). In both CA1 and CA3 regions of the dorsal hippocampus, the supressant effect of MK-801 on NMDA-induced activation of pyramidal neurones was more than 5 times greater than its effect on kainate- and quisqualate-induced activations. Haloperidol (2 mg/kg, i.v.) markedly potentiated the supressant effect of MK-801 on the three excitatory amino acid-induced activations.

These results confirm the selective antagonism of NMDA by MK-801 and suggest an anesthetic interaction between the PCP and o-halo-derivative subtypes of receptors.
EVIDENCE AGAINST THE CO-TRANSMITTER ROLE OF GLUTAMATE

In previous studies we have shown that injections of a number of excitatory amino acid (EAA) antagonists into the median raphe nucleus (MR), but not adjacent structures, result in marked increases in locomotor activity and food and water intake. In the current experiments, we attempted to clarify the receptor subtypes responsible for these effects.

Locomotor activity was measured after injections of a number of selectively competitive NMDA antagonists into the MR, and the threshold doses for the production of hyperactivity were correlated with the occurrence of some of these compounds of the NMDA receptor.

The following compounds were examined, in order of behavioral potency: CPP > DL-AP5 = DL-AP7 > Asp-AMP > Glu-AMP > AP6. In contrast, L-AP5, which have very low affinity for the NMDA receptor, were without effect. In contrast, intra-MR injections of the noncompetitive NMDA antagonists PCP and MK-801 were without effect on activity. One explanation of this finding is that NMDA receptors in the MR may not be coupled to PCP receptors. Feeding and drinking in sated rats could also be elicited by injections of the specific NMDA antagonists CPP and AP5, with the former compound being considerably more potent.

Hyperactivity, and feeding, could also be elicited by intra-MR injections of the kainate/quisqualate antagonists pBB-PZDA and GAMS at doses far lower than those expected from their affinity for the NMDA receptor. These results suggest that both NMDA and kainate/quisqualate receptors may play a role in MR function.

The GLUT content of rat cortex can be influenced by lesions of the substantia nigra. While the occurrence of large amounts of GLUT in isolated cholinergic terminals is likely to be due to contamination with glutamine-ergic synaptosomes (Supported by the MHC of Canada.)

Evidence for this view is provided by the following observations. The GLUT content of rat cortex can be influenced by lesions of the substantia nigra. While the occurrence of large amounts of GLUT in isolated cholinergic terminals is likely to be due to contamination with glutamine-ergic synaptosomes (Supported by the MHC of Canada.)

Comparative actions of excitatory amino acids on striatal dopamine and enkephalin release. B.B. Buzics, D.W. Glow and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, N6K 3R6.

In the present study, the effect of L-glutamate (L-Glu), N-methyl-D-aspartate (NDMA), kainate (KA) and quisqualate (Quis) on the release of endogenous dopamine (DA) and methionine-enkephalin (ME) from slices of the rat caudate-putamen were investigated. L-Glu, NDMA, KA and Quis, in the absence of Mg2+, produced a dose-related increase in DA release. DA release induced by L-Glu and NDMA was Mg2+-sensitive and antagonized by D-2-amino-7-phosphonoheptanoate (D-AP5) (0.5 mM). In contrast to their excitatory actions on DA release, L-Glu, NDMA, KA and Quis, in the absence of Mg2+, had little effect on ME release. L-Glu (10 nM) also failed to influence the 25 mM K+-evoked release of ME. However, in the presence of Mg2+, the 25 mM K+-evoked ME release was significantly greater than that observed in the presence of Mg2+. D-AP5 (0.5 mM) had no effect on the 25 mM K+-induced ME release in the absence of Mg2+. These results show that activation of excitatory amino acid receptors causes an increase in the release of DA, but no apparent increase in the release of ME.

(Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation)
382.1

IgG FROM PATIENTS WITH LAMBERT-EATON SYNDROME INHIBITS CALCIUM-ACTIVATED POTASSIUM CURRENTS IN LOCUST MUSCLE. G. L. Kin, Dept. of Neurology and Biomedical Engineering, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Recent evidence suggests that the antigenic target of the Lambert-Eaton syndrome (LES) IgG is a voltage-dependent calcium channel (Kin and Neher, Science 239:405, 1988). The aim of present study is to determine whether LES IgG also modifies Ca2+ -dependent potassium channel currents (I\textsubscript{K(Ca)}) of nerve terminals. The study involved 20 LES IgG patients with motor end plate clusters, and 20 control patients.

The channel currents were recorded from frog muscle fibers (BRL 34915) and guinea pig cardiac muscle (PIN). The actions of BRL and PIN in cardiac cells are well understood. Thus, we decided to use whole-cell patch-clamping techniques to study the electrophysiological actions of these agents in papillary muscles and isolated ventricular myocytes of the guinea pig. Both BRL (10^{-6} M) and PIN (10^{-6} M) caused marked shortening of action potential duration (APD) measured at 50 and 90% repolarization; APD was shorter by 26% and 41% respectively. RP hyperpolarized several mV in the presence of BRL and PIN, with little effects on other parameters. The inhibition of APD by both compounds was completely reversible in a dose-dependent manner by addition of 10^{-6} M glyburide, a sulfonylurea known to inhibit ATP-regulated K+ channels. In isolated myocytes, BRL (5x10^{-5} M) significantly hyperpolarized RP in (1-10 mV/K+) and increased the slope of the relationship between RP and K+ from 41 to -55 mV, suggesting an increase in K+ permeability. Whole cell voltage clamp experiments failed to find an increase in inward rectification. These results suggest that different mechanisms or channels may underlie the hyperpolarizing and the APD shortening actions of BRL and PIN and that the latter may be mediated through the ATP-regulated K+ channel.

382.2


Activation of K+ channels of vascular smooth cells with resultant hyperpolarization of the resting potential (RP) is thought to underlie the mechanism of action of the new vasodilators BRL 34915 (BRL) and pinacutin (PIN). The actions of BRL and PIN in cardiac cells are not well understood. To this end, we investigated whether LES IgG also modifies Ca2+ -dependent potassium channel currents (I\textsubscript{K(Ca)}) of nerve terminals. The study involved 20 LES IgG patients with motor end plate clusters, and 20 control patients.

The channel currents were recorded from frog muscle fibers (BRL 34915) and guinea pig cardiac muscle (PIN). The actions of BRL and PIN in cardiac cells are well understood. Thus, we decided to use whole-cell patch-clamping techniques to study the electrophysiological actions of these agents in papillary muscles and isolated ventricular myocytes of the guinea pig. Both BRL (10^{-6} M) and PIN (10^{-6} M) caused marked shortening of action potential duration (APD) measured at 50 and 90% repolarization; APD was shorter by 26% and 41% respectively. RP hyperpolarized several mV in the presence of BRL and PIN, with little effects on other parameters. The inhibition of APD by both compounds was completely reversible in a dose-dependent manner by addition of 10^{-6} M glyburide, a sulfonylurea known to inhibit ATP-regulated K+ channels. In isolated myocytes, BRL (5x10^{-5} M) significantly hyperpolarized RP in (1-10 mV/K+) and increased the slope of the relationship between RP and K+ from 41 to -55 mV, suggesting an increase in K+ permeability. Whole cell voltage clamp experiments failed to find an increase in inward rectification. These results suggest that different mechanisms or channels may underlie the hyperpolarizing and the APD shortening actions of BRL and PIN and that the latter may be mediated through the ATP-regulated K+ channel.

382.3


In Drosophila muscle, at least four macroscopic potassium currents can be distinguished by their differences in kinetic and pharmacological properties and by their sensitivity to a number of mutations. Studies of single channel events underlying these currents have only recently been undertaken.

In this study the single channel currents were recorded in situ in excised inside-out patches of mouse spinal cord neurons and rat ventricular myocytes using the high sensitivity method of Marty and Neher (J. Physiol. 367:117, 1985) in 22 LES IgG-treated cells. I\textsubscript{K(Ca)} was 178 pA/pF, declining by 51% from the control (358 pA/pF, n=22 cells) and 55% from the normal untreated (398 pA/pF, n=17). By comparison, I\textsubscript{K(Ca)} elicited at -80 mV fell by 20 and 11%, respectively, relative to the control and normal. These results provide evidence for a significant inhibition of Ca2+ -activated K+ channel currents in this disorder. Clinical implication of this finding is yet to be demonstrated (Supported by NIH grant NS18607 and an MDA research grant).

382.4

RYANOGENE MODIFIES THE ION-SELECTIVE AND ION-TRANSPORT PROPERTIES OF LOCUST MUSCLE POTASSIUM CHANNELS. E. Gorczynska*, P.L. Huddie* and P.N.R. Usherwood*, Department of Zoology, University of Nottingham, Nottingham NG7 2RD.

Giga-ohm seal patch clamping of adult locust muscle revealed 3 types of K+ channel, which differed with respect to the voltage and kinetic properties of channel gating and relative open channel conductances (100pS, 25pS, 10pS). A pka/pk of 0.067 was estimated (using the Goldman-Hodgkin-Katz equation) for the two largest channels. At concentrations of 5 10^{-6} M ryanodine (a plant alkaloid) increased pka/pk to a value approaching unity. The ion selectivities and internal transport properties of these potassium channels have been investigated further in the absence or presence of ryanodine using excised inside-out patches of muscle membrane with a variety of cations, viz. Li, Na, K, Rb, Cs. The relative permeabilities of these ions in the presence and absence of ryanodine will be described.

The significance of these data in terms of the molecular properties of the potassium channels and their transport of cations will be discussed.

382.5

PHARMACOLOGICAL CHARACTERIZATION OF SINGLE-CHANNEL K+ CURRENTS IN DROSOPHILA MUSCLE. M. Gorniak and C.P. Usherwood*, Dept. of Biology, Univ. of Iowa, Iowa City, Iowa 52242.

In Drosophila muscle, at least four macroscopic potassium currents can be distinguished by differences in their kinetic and pharmacological properties and by their sensitivity to a number of mutations. Studies of single channel events underlying these currents have only recently been initiated in muscle membrane vesicles and cultured myotubes. We now report single channel currents observed in body wall muscles in situ in wild-type larvae.

Using the inside-out patch clamp configuration, we have observed several distinct types of single channel. The most prominent one was a calcium-dependent, becoming activated in the concentration range of 10^{-7} to 10^{-5} M. The channel displayed a very high open probability at 10^{-5} M and a pKa of 6.85 (in symmetrical 130KCl), and was not sensitive to 50mM TEA, 1mM 4A-P, and 1mM quinidine. It was blocked by barium at concentrations greater than 0.1mM. A second channel type (pKa=8.55), with a conductance of 12pS in 50mM 4A-P, was not affected by barium but was blocked by 1mM 4A-P. A third channel type shared the pharmacology of the 12pS channel type and had a conductance of twice as large. Barium caused a slow block in these three channel types whereas TEA and quinidine led to a fast block. A fourth channel type was highly sensitive to TEA, being almost completely blocked at 1mM. We are currently attempting to correlate these single channel conductances to the previously described macroscopic currents.

382.6

OPEN CHANNEL NOISE IN CALCIUM-ACTIVATED POTASSIUM CHANNELS. M.I. Slavinsky*, Dept. Anesthesia Research, Physiology and Biomedical Engineering Unit, McGill University, Montréal, Québec, Canada.

The current through an open ion channel is carried by discrete ions and is thus expected to show fluctuations due to a 'shot noise' (Schottky, Ann. Phys. 57, 541-567, 1918), and as recent studies in ACh receptor channels (Sigworth, Biophys. J. 47, 709-720, 1985) have shown due to a 'shot noise' (Schottky, Ann. Phys. 57, 541-567, 1918), to arise from fluctuations in the conformation of channel protein also. The variance of the open-channel current exceeds that of shot noise cannot account for the difference. The low-frequency component of the power spectrum (corrected for the background noise and transfer function) reveals that : 1) whole cell (L-type) Ca currents are not obviously altered the Ca^{2+} sensitivity of K(Ca) channels in excised inside-out patches. Supported by NIH NS18788.
POTASSIUM CHANNELS II

by the blockers which affected the K+ current. Less frequently we observed potentials more negative than -70.

activating or inactivating (during a 500 ms voltage step) inward currents at indicating potassium selectivity. Many cells had slowly activating outward currents when cells were held at -30 mV. It was abolished by 500 μM quinidine and was potentials for this current, as measured by tail currents in 10 cells, were -70 step. This current exhibited steady-state inactivation, being much reduced or absent depolarization beyond about -30 mV, which inactivated during a 500 ms voltage

technique. Cells were dispersed with papain and plated onto glass cover-slips for under constant flow of solution bubbled with 95 % O2 - 5%

Polscher sag corresponding to decreased membrane resistance. This inward rectification was present in Krebs’ solution containing choline substituted for K+ and Na+. The inward rectification was absent in Krebs’ solution containing choline substituted for K+ and Na+. The inward rectification at potentials negative to -80 mV was potential of about -60 mV. The persistent potassium current which was already activated at the resting

Supported by NIH grant NS5134 (DKH) and a grant from the Ida Russell Cades fund (BRJ).
382.13  

Using whole-cell voltage-clamp techniques we identified two distinct transient outward currents in hippocampal neurons acutely dissociated from adult guinea pig or tissue cultured from fetal rat. In control saline with 10-20 mM TEA, depolarizing steps from relatively hyperpolarized holding potentials elicit a rapidly-activating A current, which inactivates fully with a time constant of 60-100 ms. 4-AP (1-10 mM) reduces total transient current, unmasking a component which inactivates more much more rapidly. 10-20 ms. The 4-AP-insensitive transient current is completely blocked by 100-200 µM Cd or by low Ca saline. Conversely, addition of Cd to the control saline reduces total transient current and slows the time constant of inactivation. In Cd, the residual transient current, corresponding to the A current (IA), was blocked by bath application of 4-AP. Both transient current components showed similar voltage-dependence.

The contribution of IA and the Cd-sensitive current to total transient current was variable. IA was ubiquitous and invariably larger than the Cd-sensitive transient in acutely isolated adult cultures. Blockade of IA by Cd was large and more prevalent in cultured neurons from fetal rat hippocampus.

382.14  
**SINGLE ELECTRODE WHOLE CELL VOLTAGE AND CURRENT CLAMP ANALYSIS OF POTASSIUM CHANNELS IN HAIR CELLS ISOLATED FROM THE CRISTA AMPULLARIS OF THE FROG.**  C.D. Hosley1, C.D. Norris1, and P.R. Farrar. Dept. Pharmacology & Otolaryngology, Tulane University, New Orleans, LA 70112.

Hair cells from the crista ampullaris of the frog (*Rana pipiens*) were isolated enzymatically (0.17 mg/ml papain in low Ca2+ artificial perilymph, placed in short-term culture for 2-3 days in L-15 medium; Sigma). The resting membrane potentials (Vr) were -44.9 ± 1.5 mV (x ± SEM; n = 51). Two types of outward current were identified corresponding to the fast transient K+ current (IK) and the slower Ca2+ dependent K+ current (IKCa). The transfer of the K+ channels inhibits time constant of about 16 ms. Tetraethylammonium (TEA; 10 mM) blocked only the IK current. Replacing the K+ (103 mM) with equimolar Cs+ in the internal solution abolished the outward current and left only a small inward current. The mean IK and IKCa conductances (from -20 mV to -30 mV) were 2.1 ± 0.1 and 0.6 ± 0.1, respectively (n = 51). Injecting 1 nA of current produced damped oscillations in cell membrane potentials. These results previously reported in hair cells of putative auditory function (Crawford and Fettplace, J. Physiol. 312:377-412, 1981; Lewis and Hudspeth, Nature 304:538-541, 1983; Picciotto and Ashmore, Hear. Res. 27:75-83, 1987), is thought to be the result of an interaction between inward Ca2+ current and the outward IKCa current. TEA blocked this response. Supported by N.H.I. NS 22031, Southern Heart & Speech Fund; N.Z.DRF, MRC (N.Z.).

382.15  
**COMPARISON OF 4-AMINOPYRIDINE (4-AP) AND TETRAETHEROAMINOACRIDINE (THA) ON MEMBRANE PROPERTIES AND IONIC CURRENTS IN GUINEA PIG BASAL FOREBRAIN.**  J.A. Sim* and W.H. Griffith (Spon: L.L. Keeley). Dept. of Medical Pharmacology and Toxicology, College of Medicine, Texas A & M University, College Station, TX 77843.

The actions of 4-AP and THA were studied in basal forebrain (BF) neurons in slice using intracellular recording and single electrode voltage-clamp (SEVC) techniques. Both compounds decreased several presumed potassium (K+) conductances, but over different concentration ranges. In all cell types, 4-AP (100-300 µM) hyperpolarized the membrane potential, increased membrane conductance, prolonged action potential duration and increased total transient current. In contrast, THA (1-100 µM) had no apparent effect, except at high concentrations (4-AP) or in some neurons (THA). 

Our results demonstrate numerous differences between 4-AP and THA when compared in BF neurons. Supposed by NIH Grant NS22456 (WHG) and the U.K. Medical Research Council (JAS).

383.1  
**THE INFLUENCE OF INTERRMITTENT ELECTRICAL STIMULATION ON THE BIOCHEMICAL PROPERTIES OF A PREDOMINANTLY FAST MUSCLE IN ADULT AND SENESCENT FISCHER-344 RATS.**  T.J. Walters*, T.J. Farrar, T.J. Farrar, and H.L. Sweeney. Dept. of Kinesiology, Institute for Neurological Sciences Research, Univ. of Texas, Austin, TX 78712.

The influence of elevated neural activity on the biochemical properties of the predominantly fast flexor digitorum longus (FDL) was investigated in adult (7-8 mo.) and aged (12-16 mo.) Fischer-344 rats using in vivo single electrode whole cell voltage and current clamp studies. The FDL was stimulated via an electrode cuff around the tibial nerve at a frequency of 10 Hz for 8 hr/day for periods ranging from 0-90 days (0, 20, 35, 60, and 90 days). The tibial nerve displayed an increase in the twitches with tetanic tension (P0.5) following 20 days of stimulation, with no significant increase at subsequent time points. The increase in P0.5 was the result of both an increase in P0 and a decrease in P0. There were no significant differences between the two ages at any of the corresponding time points. A significant increase occurred in the time to peak twitch tension occurred by 20 days in both ages (14.03 ms vs. 17.60 ms) and by 30 days in adult rats (15.34 ms vs. 17.64 ms). No subsequent significant increase in TTP occurred after these differences between any of the corresponding time points between the two ages. The maximal velocity of unloaded shortening (Vmax) was determined using the slack test. Although an increase in Vmax occurred at 90 days in both adult rats (248 mm/sec vs. 211 mm/sec) and aged rats (232 mm/sec vs. 169 mm/sec), this increase was not statistically significant. The fatigue resistance of the FDL was determined using the Brooke fatigue test. A progressive significant increase in fatigue resistance occurred through 30 days of stimulation in both ages, with no significant increase occurring thereafter. The fatigue resistance of the predominantly fast FDL remains remarkably plastic in response to an extreme elevation of neural activity through very old age in Fischer-344 rats.

383.2  
**THE INFLUENCE OF INTERRMITTENT ELECTRICAL STIMULATION ON THE BIOCHEMICAL PROPERTIES OF A PREDOMINANTLY FAST MUSCLE IN ADULT AND SENESCENT FISCHER-344 RATS.**  R.P. Farrar, T.J. Walters, and H.L. Sweeney. Dept. of Kinesiology, Institute for Neurological Sciences Research, Univ. of Texas, Austin, TX 78712.

The plasticity of muscle has been inferred to decline during senescence. Previously we (Cartee and Farrar, 1987) demonstrated that the gastrocnemius of young and old rats given the same aerobic stimulus imposed by running for an hour per day, at the same absolute workload responded with the same absolute increase in aerobic capacity. The purpose of this study was to determine whether an imposed neural stimulus, to a muscle with predominantly fast motor units would induce similar changes both in aerobic capacity, and fiber type in young adult and senescent rats. We imposed an alarmed stimulus to the plantar muscles in adult (7-8 mo.) and aged (27-28 mo.) Fischer-344 rats by stimulating the sciatic nerve at a frequency of 10 Hz for periods ranging from 0-90 days (0, 20, 35, 60, and 90 days). The mass of the plantar muscle in both adult and aged rats decreased 20-25% following 20 days of stimulation and did not change significantly thereafter. The aerobic capacity, as measured by the climbing ability, of the plantar muscles increased 3-6 fold by day 20 of stimulation and dropped down to approximately a 2-fold increase by day 35 of stimulation and remained there throughout the remaining periods of stimulation. There was not a shift in fiber type, as determined by gel electrophoresis on native myofibrillar proteins, until 60 days of stimulation. The shift in fiber type occurred in the fast population with an increase in ffa fibers and concomitant decrease in llb fibers of approximately 20%. The time course of change in either aerobic capacity or fiber type did not vary between adult and aged rats. These data demonstrate that the plantar muscle, which has predominantly fast motor units, maintains the same degree of plasticity in the senescent rat as that of young adult rat, when a neuronal stimulus, similar to that of slow muscle, is imposed for up to 90 days.

Four adult female cats had the lumbar region of their spinal cord functionally isolated by transecting the cord at T12-L1 and at L7-S1 and cutting all dorsal roots bilaterally between these two cord segments. The cats were maintained in excellent health for 6 months. One limb from each cat was passively exercised through a range of motion mimicking a step cycle for 30 min/day, 5 days/week. During the week prior to physiological testing, two 24-hour EMG recording sessions were used to verify that the muscles in the lower limb were virtually silent and recordings from a force transducer on the soleus tendon showed that up to 1 kg of force was generated during the passive exercise. In 3 of 4 cats, the soleus muscle in both limbs was significantly larger (20-30%), had a Po that was 28-42% higher and, although more variable, a 20-60% lower Vmax than the soleus in the unexercised limb. All other parameters were similar in the two legs.

Adaptations in the muscle atPase activities and isoforms patterns were generally consistent with those in Vmax. These data indicate that short periods of cyclical passive stretching that results in a significant amount of force at the muscle tendon may have a beneficial effect in maintaining the mass and the mechanical properties of electrically silent mammalian muscles. Supported by NIH Grant NS38875, NB36651, NB24752 and the NEA.

383.5 RECOVERY OF FORCE PRODUCTION AND FORCE SENSATION FROM FATIGUE. D.W. Gallaway and L.E. Larsson. The Ohio State University, Dept. of Psychology, Columbus, OH 43210.

Our purpose was to compare the recovery time course of force production and force sensation with the recovery time course of force sensation following fatigue. Ten subjects participated after giving their informed consent. During each experimental session fatigue was produced in the right anterolateral thigh muscles by holding 50% of maximal voluntary contraction force (MVC). This contraction ended when force fell to 20% MVC. In half the experiments a pressure cuff was inflated around the fatigued leg during recovery. The recovery of force production was assessed with: 1) periodic MVC’s and 2) the EMG-force ratio during submaximal contractions. Force sensation measurements at 20-50% MVC were obtained using the matching technique (Cannon, R. and E. Cafarelli, J. Appl. Physiol., 53:635-642, 1982). In the non occluded experiments, MVC recovered to 80% of control after 3 min but no further. In comparison, force sensation recovered to control after 1 min. Occclusion was removed there was a gradual increase in force producing capacity and a more rapid decrease in force sensation. These results indicate that force sensation recovers somewhat independently of force production and responds to central feed-forward information during recovery from fatigue. Supported by NSERC A6633.

383.6 STERNOCLEIDOMASTOID MUSCLE INHIBITION INDUCED BY TRIGEMINAL REGION STIMULATION. P.D. Brown*, C.T. Clark and M. Natke*, UCLA Dental Research Institute, Los Angeles, CA 90024 & Children’s Hospital and Chapman College, School of Physical Therapy.

Research suggests a high degree of coupling between trigeminal and cervicocervical innervated muscles. This study documented the presence of co-inhibition in the sternocleidomastoid (SCM) and masseter muscle when using mechanical and painful electrical stimuli applied to the oral and perioral region. Electromyographic activity of the masseter and SCM muscles was recorded bilaterally with surface electrodes during the following conditions: maximal clenching and maximal neck flexion or extension. The inhibition taps, electrical stimulation intraorally to the anterior maxilla gingiva or extraneously to the skin over the mental nerve. The subjects were instructed to voluntarily inhibit and the SCM inhibition of similar timing with mental tap. It is very likely that this inhibition represents a response of the SCM to a nerve that was caused by the direct mechanical perturbation from the tap. In contrast, SCM inhibition with electrical stimulation though often elicited, was not a consistent response that was elicited at all subjects nor in every trial of subjects in whom it was elicited. The SCM inhibition was similar in timing and character inhibition (e.g., it was frequently a double silent period).


Single fibers from the thin transversus abdominis muscle of the garter snake were identified by physiological criteria as either twitch (T) or tonic (T) (Wilkinson and Lichtman, J. Neurophysiol., 1985). Four different single fibers (T, shown in receptor 1) were used to perform microanalyses for glycolytic (lactate dehydrogenase), oxidative (malate dehydrogenase, fumarase, H- and NADH-glycerate dehydrogenase) and high energy phosphate (adenylkinase, AM enzymes or 2) denaturing SDS discontinuous PAGE for myosin heavy chain conditions and fiber type. Fibers had high glycolytic and CK activities, and contained one myosin heavy chain isoform similar to rat fast (IIB) myosin. F fibers had high glycolytic and oxidative activities, intermediate AK activity, and expressed two myosin isoforms, one similar to rat slow (1) and the other to rat fast. T fibers had high oxidative activity with low levels of AK; they expressed a single myosin isoform similar to rat slow. The three fiber groups distinguished by metabolic criteria match those of the rat hindlimb; the same groups are distinguished by myosin isoform composition. Thus, energy generating enzymes and myosin isoforms strictly correspond in this reptilian muscle. Supported by NIH grants M38375, NA36651, NB24752 and the NEA.


Forces developed by single motor units have been studied in reduced preparations (by stimulation of either motorneuron cell bodies or axons), and in humans (by sphenoid averaging techniques). Detailed information on the mechanical behavior of SC units in motor units is available for isoformic conditions only. We set out to study the dependence of motor unit twitch and tetanic forces on both muscle length and rate of change of muscle length.

The medial gastrocnemius muscles of 15 cats anesthetized with pentobarbital were separated from the surrounding tissues, and the muscle was attached to a force-multiplying unit with a controlled muscle stretcher through a custom-built force transducer. Precautions were taken to prevent the tendons from drying out. All other hindlimbs muscles were denervated. Laminectomies were performed to functionally isolate it from all other muscles with spliting either the L7 or T11 ventral roots. With this set-up, motor unit twitch forces and tetanic at various frequencies were studied at different muscle lengths and movements at velocities of 50 and 100% and a 10% MVC. The method involved comparing the amplitude of a reference H-reflex (Href) with that of a H-reflex preconditioned by recurrent inhibition (HI). R-reflexes were elicited in the soleus muscle by stimulation of the tibial nerve. Fatigue was confirmed by a 20% drop in MVCs performed periodically during the contraction. The test of recurrent inhibition revealed relatively greater reductions in Href than Href for 5 subjects in the constant torque condition and 3 subjects in the constant weight condition. For these subjects, Href values dropped by 10% MVCs within 10 vs 20-30% MVCs within 20-50's of voluntary contraction. The greater drop of Href vs Href when voluntary am. is constant or declining indicates increasing recurrent inhibition to the soleus motorneuron during sustained contraction. Supported by NSERC.
MODULATORY EFFECT OF ZERO SODIUM MEDIUM ON CONTRACTION OF BUCCAL MUSCLE OF APlysia. J. Daube*: J.M. Anderson*, M.J. Cadow*: S. Patel*, & M. Harris*. Dept of Physiology, Wayne State Univ., Detroit, MI 48201. ACh depolarization was both sodium- and calcium-dependent. ONa hyperpolarized muscles 2-6 mV and reduced ACh depolarizations to 20%-50% of control. ACH depolarization recovered within seven min in normal medium (ASW). ACh elicited contractions were reduced in ONa. Upon return to ASW, ACh contractions partially recovered within 2 min and were subsequently potentiated. Maximal potentiation after 1, 2, & 10 min in ONa occurred within 4 min of returning to ASW and were 130 ± 10% (n=15), 140 ± 10% (n=15), & 600 ± 130% of control, respectively (p<.05). Time constants for recovery were about 10 min. ONa (10 min) increased Ca45 influx from 350 ± 70 (n=7) to 1356 ± 200 (n=7) cpm/mg protein (p<.05, t test). ONa may cause Ca influx via Na-Ca exchange. Additional Ca in sarcoplasm or sarcoplasmic reticulum could enhance responses to Ca entering in response to ACh. Saponin-skinned Aglysia muscle fibers contracted in response to Ca. Raising the "background" level of Ca in perfusion medium increased the contractile response to Ca pulses, thereby modeling the response of propagating contraction. (supported by MDA and NIH grant RR-08167).

INTERSPECIES COMPARISON OF CARDIAC MUSCLE GANGLIOSIDES. K.G. Isaksson* and L.A. Czay*. Dep. Anat. Sci. & Neurobiol., University of Louisville, Louisville, KY 40292. Ganglioside content of hearts from a number of different species was examined, since studies of glycolipids in non-neuronal, electrically excitable tissues have been rare. Adipose tissues were removed and extracted gangliosides were fractionated into mono-, di-, and oligosialosyl forms on DEAE-Sephadex. The highest content of di- and oligosialosyl gangliosides was found in the hearts of eel, tuna and sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and sheep, horse and pig; human values were slightly lower.

MODULATORY EFFECT OF ZERO SODIUM MEDIUM ON CONTRACTION OF BUCCAL MUSCLE OF APlysia. J. Daube*: J.M. Anderson*, M.J. Cadow*: S. Patel*, & M. Harris*. Dept of Physiology, Wayne State Univ., Detroit, MI 48201. ACh depolarization was both sodium- and calcium-dependent. ONa hyperpolarized muscles 2-6 mV and reduced ACh depolarizations to 20%-50% of control. ACH depolarization recovered within seven min in normal medium (ASW). ACh elicited contractions were reduced in ONa. Upon return to ASW, ACh contractions partially recovered within 2 min and were subsequently potentiated. Maximal potentiation after 1, 2, & 10 min in ONa occurred within 4 min of returning to ASW and were 130 ± 10% (n=15), 140 ± 10% (n=15), & 600 ± 130% of control, respectively (p<.05). Time constants for recovery were about 10 min. ONa (10 min) increased Ca45 influx from 350 ± 70 (n=7) to 1356 ± 200 (n=7) cpm/mg protein (p<.05, t test). ONa may cause Ca influx via Na-Ca exchange. Additional Ca in sarcoplasm or sarcoplasmic reticulum could enhance responses to Ca entering in response to ACh. Saponin-skinned Aglysia muscle fibers contracted in response to Ca. Raising the "background" level of Ca in perfusion medium increased the contractile response to Ca pulses, thereby modeling the response of propagating contraction. (supported by MDA and NIH grant RR-08167).

MEASUREMENT AND FOCUSING OF MAGNETIC STIMULI APPLIED TO NEURAL STRUCTURES. R.C. Bilderback*, F. Portescu*, A. Fisher*, & S. E. Elsberry Lab of Neurophysiology, University of California at San Diego, 92037 The high frequency of stimuli (one stimulus per 100 microseconds) employed in magnetic stimulation of the nervous system (nerves, spinal roots and cerebral cortex) can cause fatigue and induce a "skin effect" when applied to a metal surface. We have employed the induced field on the inside of a copper cone to compress and concentrate the magnetic field produced by the coil of a magnetic stimulator (e.g. Cadwell). This allows the production of a highly concentrated field at several centimeters from the coil producing the focusing of the field at its exit. Tests were carried out using a 1 cm current loop connected to a peak reading voltmeter calibrated in kilogauss (designed by Dr. Portescue). In one such test the field at 5 cm from the coil read 0.4 Kgauss at 5 cm (at apex). When a 16 gauge copper cone (with a diagonal saw cut to prevent current flow) was put in place centering on the magnet coil, the reading was increased to 1.4 Kgauss indicating a tenfold concentration. The clinical features of the focussed field was investigated in the isolated frog muscle and by application to a subject's cutaneous nerves (ulnar, median and digital). EMGs resulting from the experiments will be shown.
384.1
DEVELOPMENT OF LIMB TRAJECTORY FORMATION. D. L. Claman and J.M. ABBS. Dept. of Neurology & Waisman Center, University of Wisconsin 53705.

Although previous studies of reaching movements indicate invariances in spatial and temporal trajectory properties that are strongly influenced by visual information, little is known about the development of these properties.

Visualy elicited planar arm movements were studied in 5-14 year old children. Movements were recorded by sliding a stylus along the surface of a digitizing tablet to various LED targets. In one experimental condition the target remained lighted for the duration of the trial and in the other the target extinguished at movement onset. Further, we presented an analysis of the time scaling effects of task performance on pain perception little is known about the development of these properties.

The process of trajectory formation and the use of visual information in movement both appear to develop in a slow and orderly fashion through the ages 5-14, at which point more adult-like patterns are seen. Developing adult abilities to use visual information and to scale movement size and speed may underlie the performance of many motor and perceptual tasks. Supported by NIN grants (5R-12747 & RO-03392).

384.2

The contribution of the eye movement to the guidance of the arm not only depends on a pure visual information, but also on extraretinal factors (Mather, J. and Fisk, J. Exp. Psychol. 37A:315-318, 1985). A study of these factors was conducted on right handed, head fixed human subjects sitting in front of a set of LED's aligned in the horizontal plane, in the left hemifield. They were asked to move a handle, with the right hand, in the direction of one of the LED's, without visual feedback about the arm movement. In one series of experiments, the subjects kept their eyes centered; in another, they oriented their eyes, together with their arm. The target was either continually on (visual condition) or turned off three seconds before the onset of the movement (non-visual condition).

The relation of the arm final position error with eccentricity was different in eye fixed and eye mobile conditions. The characteristic of the arm error distribution were comparable in visual and non-visual conditions. The fact that eye movement, in these conditions, does not globally improvements of accuracy, though it modifies its characteristics, suggests that the extraretinal factors reflect mechanisms different from a simple substitution of vision.

384.3
THE ROLE OF COMPLIANCE IN CONSTRAINED BALLISTIC ARM TRAJECTORY FORMATION. D.L. Claman and J.M. ABBS. Dept. of Neurology & Waisman Center, University of Wisconsin, Madison, WI. 53792.

Previously we have argued that a joint level kinematic planning strategy accounts for the curvature of ballistic arm movement data. In particular, we consistently observed curved trajectories when a movement between two target pairs planned when it is constrained (by a light switch) to be a straight line. We hypothesized that the trajectory is commanded as though the constraining wall were not there, and the natural compliance of the arm is relied upon to follow the constraint surface. This hypothesis predicts that the stiffness component of the compliance should produce an envelope of forces on the wall that resembles the unconstrained hand path.

Further, we present an analysis of the time scaling property of dynamics which indicates that the differences between forces on the wall in the forward (upward) and reversed direction of movement can only be due to the viscous (passive and active velocity feedback) component of the compliance. In fact, together with our hypothesis, reasonable assumptions about the arm viscosity allow the prediction that the upward and downward forces on the wall should only be equal at the elbow joint reversal point. All of these predictions are found to agree with the data. In addition, simulations qualitatively produce the experimental force envelopes.

384.4

Although a growing body of research examines the effects of task performance on pain perception little is known about the effects of pain on motor performance. While movements executed to lighted targets yield faster movement speed and size observed in adults for this task the target extinguished at movement onset. Although overestimation of targets overshoot the target. Although overestimation of targets overshoot the target, this hypothesis predicts that the stiffness component of the compliance should produce an envelope of forces on the wall that resembles the unconstrained hand path. Further, we present an analysis of the time scaling property of dynamics which indicates that the differences between forces on the wall in the forward (upward) and reversed direction of movement can only be due to the viscous (passive and active velocity feedback) component of the compliance. In fact, together with our hypothesis, reasonable assumptions about the arm viscosity allow the prediction that the upward and downward forces on the wall should only be equal at the elbow joint reversal point. All of these predictions are found to agree with the data. In addition, simulations qualitatively produce the experimental force envelopes.

384.5
WITHDRAWN

384.6
EFFECTS OF AGONIST MUSCLE LOAD ON MOST-RAPID ISOMETRIC FORCE PULSES. B. Leglaud*, M. Wiersbicka*, D. Crou* and B. Shaban. Massachusetts General Hospital and New England Medical Center, Boston, MA.

Contraction time (CT)of most-rapid isometric force pulses in intrinsic hand muscles has been reported to be independent of agonist muscle load (AL) prior to contraction. (Freud and Budgoen, 1978). We investigated the dependence of CT and EMG parameters on AL in most-rapid contractions of elbow flexor muscles to determine if the CNS motor program subserving most-rapid force pulse generation changes according to AL. With visual feedback of the force signal, elbow flexors were isometrically loaded in 5 normal men, ages 25-38, to 0.20, 0.40, 0.60, 0.80, and 1.00 of their maximum voluntary force (MVF). At each AL, 10 most-rapid isometric force pulses were made to the same target: 201 MVF. In 4 out of 5 subjects, peak CT and burst duration (BD) were relatively constant only for AL <60% MVF. At AL >60% MVF, CT and BD significantly increased. In all 5 subjects, the integrated EMG burst area was unchanged. To generate most-rapid elbow flexor contractions of given amplitude under lower ALs, the CNS scales agonist EMG activity proportionally to the load presumably by modulating motorneuron (MN) firing rate and possibly the number of MNs recruited. At highest loads, duration of MN discharge is scaled to the load, perhaps because the limits of MN firing rate and recruitment mechanisms have been reached.
CHARACTERISTIC PATTERNS OF RAPID POSITIONING: ACCURACY FOLLOWING POSTURAL ISOMETRIC CONTRACTION. L. Enyvns, O. Dry*. Human Performance and Health Sciences Dept. Rice University, Houston, Texas 77251.

The purpose of this study was to compare post-contraction positioning accuracy and electromyographic (EMG) patterns of biceps and triceps brachi muscles during rapid elbow flexion and extension movements to a target. Following a learning session 10 subjects performed twenty control trials without augmented feedback. Control trials were performed following the subject preceded each movement with a 3 x isotropic contraction of the antagonist muscle used for the subsequent movement. Separate analyses of variance revealed significant undershooting in positioning accuracy for both flexion and extension post-contraction movements compared with control conditions. Integrated EMG (IFEM) of the antagonist muscle was significantly less in the experimental condition than in the control condition for both tasks, with no difference between antagonist limb. Frequency Analysis of the EMG revealed significantly higher peak frequencies in the biceps during the post-contraction flexion movement. It was concluded the undershooting following isotropic contraction was dependent on the total motor output of the agonist output and de-synchronization for flexion movements.


Humans are able without conscious effort to turn their eyes towards a skin point stimulated in the dark. To carry out this act requires combining information about the skin location of the stimulated point with information about the angles of the joints interposed between it and the head. When many joint angles and degrees of wrist, rate, direction, and involved, the underlying computations are extremely complex. We have investigated the ability of a massively parallel network to carry out these computations.

The network consists of an input layer (10 units), a hidden layer (variable number of units) and an output layer (3 units). The input units encode the angles and angle rate at joints interposed between the finger tip and the head. The three output units encode the vertical and horizontal angles of the eye and its fixation distance. By use of back-propagation, the network was trained to bring the eye to bear on the fingertip given an arbitrary posture of the arm and neck.

Several runs with training sets of different sizes (100, 1000, 10000) and random starting points were carried out. In all cases, performance of the training set became extremely accurate. Performance on a transfer set improved as the training-set size increased. The properties of hidden units were characterized by use of several multivariate approaches including cluster analysis and were analyzed qualitatively by examination of Chomoff-face and barplot ("Hinton diagram") displays. The multijoint receptive fields of units in the network form a basis for predictions regarding the properties of neurons responsible for encoding body position in brain regions including area 5.


In an effort to understand the control of multi-joint movements, we examined two and three link human arm and arm with pointer movements between targets located in a sagittal plane. The movement path, rate, direction and, as well as the inertial properties of the pointer (mass and principal moments of inertia) were varied.

The curvature of endpoint paths was found to vary with target location. Paths for three link movements were similar for movements in the horizontal plane. The inertial properties of the pointer. Paths for two link movements were qualitatively similar to comparable three link movements and were likewise independent of rate and direction. In general, the form of the tangential velocity curve of the endpoint scaled with rate and moment but not with movement direction. In many cases, the curve was positively skewed regardless of direction.

For three link movements, path and trajectory invariance of the endpoint are not sufficient conditions for joint programming. In trajectory scaling instances, invariance in endpoint space was found in the absence of joint space invariance.

The redundancy is constrained in three link movements and the dynamics of both two and three link movements will be discussed.

THE INFLUENCE OF TASK AND ORGANIC CONSTRAINTS ON INTRALIMB KINEMATICS IN A DRAWING TASK. B. E. A. van Emmerik*, K. M. Newell. Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Intra-joint and joint-stylus kinematic couplings were examined as a function of various task and organic constraints in a circle drawing task. The constraints manipulated included natural limb dominance (left versus right-handers), the use of the dominant or nondominant limb, writing orientation (horizontal versus vertical), and circle diameter. The most consistent kinematic constraints and organization were observed between left- and right-handers (9=6), although there was a higher degree of phase-locking between the joints in the nondominant limb for right-handers subjects in the horizontal orientation. This suggests that without the constraint of writing hand and right-handers adopt similar coordination functions. Correlations between the linkages significantly increased with the scaling of circle diameter, and with changing the drawing orientation from horizontal to vertical. The topological features of the stylus kinematics remained invariant under the constraints imposed, inspite of the systematic changes that occurred in the topological features of the joint motions. It is proposed that invariances and transitions in joint and stylus kinematics are a function of the task constraints that need to be optimised.
EFFECTS OF REPETITION ON ARM TRAJECTORIES DIRECTED TOWARDS MOVING TARGETS: STUDIES ON PERIPHERAL TACTILE FEEDBACK (P. T. Tator). Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Although many findings have been reported regarding directed limb movements in humans, few studies address the interception of moving targets. This study describes the strategies used to intercept a moving target and assesses modifications of performance occurring with practice. Subjects were instructed to maintain contact with an arm switch until a light signaled they could initiate movement towards a target moving along a 1 m horizontal track. The target velocity, ranging from 60 to 95 cm/sec, was unknown to the subject until the first trial. Thirty two trials each, at a different velocity, were performed for right to left motion and left to right target movements. Movement trajectories and EMG's for biceps, triceps, and brachioradialis were determined, and reaction time and movement duration for each trial were calculated. In general the spatial characteristics of the trajectory were quite subject specific and varied little over successive trials. Trajectories usually consisted of at least three components: an initiation phase marked by a movement towards and then away from the target's path; a high acceleration phase which brought the distal limb up to the speed of the target; and a final phase which was directed towards the target. During each phase the hand moved along a curved trajectory. The primary modification with practice was an increased acceleration during the second component of the movement. The EMG data revealed the recorded muscles were not involved in the first phase of the movement. The data indicate that the trajectory's envelope is subject specific and that changes in performance associated with practice do not alter the spatial characteristics of the movement. Supported by NIH grant NS21958.


Monkeys were trained to perform wrist movements in 8 different directions. For each task, the forearm was performed with the forearm in 2 positions: fully pronated or midway between pronated and supinated. We examined the patterns of activity in wrist extensors using pairs of fine wire electrodes. The electrodes were also stimulated to determine each muscle's direction of action.

When the forearm position was changed from pronated to the mid position, the direction of maximal agonist activity in all wrist extensors shifted 20-100 degrees in the radial direction. This shift occurred even though the forearm was also altered in direction of movement produced by electrical stimulation for some, but not all wrist extensors. For example, when the forearm was pronated, agonist activity in extensor carpi ulnaris (ECU) was largest for ulnar deviation. When the forearm was in the mid position, agonist activity in ECU was largest for wrist extension. This shift occurring movement though ulnar deviation was evoked by electrical stimulation of ECU in either forearm position.

These results indicate that the activity of a wrist muscle can be dissociated from: the muscle's direction of action, the direction of joint movement and the direction of movement in space. Supported by funds from the VA Medical Research Service.


This study investigated coordination of repetitive movements involving one, two or three limbs. Required tapping movements of the hand or foot in the sagittal plane performed at self-determined preferred and fast rates. As prior work (Kay et al., J. Exp. Psych. Hum. Per. 1987, 13:178-192) has shown greater phase differences (phase diff.) over altered (180° phase diff.) movements in bimanual tasks, these two coordinate modes were used in the present study for bilateral (2 limb) and interlimb (4 limb) tasks. Subjects included movements (period, amplitude, velocity) and for bilateral and interlimb tasks, measures of phase difference and dispersion. At preferred rates, tapping period and amplitude were similar to the subject's wide range of voluntary and interlimb tasks in the simultaneous mode, whereas, in the alternating mode, periods were longer and variable. No differences between hands and feet were noted on 8 seated subjects. Interlimb differences in tapping period and variability were observed between simultaneous and alternating modes. While tapping period did not differ between limbs, the tapping movement showed a phase lag of the hands in the alternating mode. For both bilateral and interlimb tasks, alternating movements were more strongly coupled (as measured by phase dispersion) than were simultaneous movements, especially for the feet. Thus, the major finding is that in interlimb coordination, alternating movements of the feet are more consistent and more strongly coupled than the hands. This coupling may utilize an endogenous neural synergy associated with locomotion as suggested by Gentile and P.A. Bates (Motor Control, 1997, 2:261-10).

THREE DIMENSIONAL TRAJECTORY ANALYSIS OF A PATIENT WITH CONGENITAL MIRROR MOVEMENTS. H. Potgieter and M. Kritchevsky*. The Salk Institute, La Jolla, CA 92038, Department of Neurosciences, UCSD, La Jolla and V.A. Medical Center, San Diego.

Mirror movements are involuntary movements executed by one side of the body in response to voluntary activation of homologous muscles. Although such movements have been described qualitatively and with surface EMG recordings, the spatial and temporal characteristics of these movements remain poorly understood. Selected simple, repetitive and complex limb movements were studied in a 20 year old woman with congenital mirror movements and no other neurological disorder. Movements were digitized in three dimensional space, reconstructed computergraphically, and analyzed numerically and graphically. Mirror movements had smaller amplitudes than did the corresponding voluntary movements and were generally slower. In general, temporal coupling between mirror and voluntary movements. Nonetheless, mirror movements were not always a perfect mirror image of the corresponding voluntary movements and sometimes differed in timing and trajectory shape from the original movement. Substantially greater mirror movements were elicited by distal as opposed to proximal movements and mirror movements were enhanced when loads were applied to the hand executing the voluntary movement. These data will be related to anatomical bases of congenital mirror movements.

CONTROL OF SINGLE- AND MULTI-JOINT MUSCLES DURING THE MAINTENANCE OF A POSTURE AT THE HUMAN ELBOW COLUMNS F. ROY and W.R. Byrnes, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

We examined the activation of muscles that act in elbow flexion/extension and wrist supination/pronation during various static postures. Intramuscular and surface EMG's for five muscles (flexion/extension, varus/valgus, and supination/pronation) have been simultaneously recorded while subjects protracted steady-state forces at the wrist. Trials were done with subjects at three different elbow flexion angles.

We found that some elbow-joint muscles that do not contribute to supination/ pronation torque generation are nonetheless varied significantly during these loads. For example, when the wrist is in a supine posture, the triceps acts as if it were a supinator--EMG's increase with increasing supination load. The load conditions being the same. However, when the wrist is held in a pronated position, the triceps mimics a pronator, increasing pronation torque. Other muscles were also significantly influenced by supination/pronation position. Biceps and pronator teres varied as might be expected, but variations in brachial muscles were unforeseen.

Since supination/pronation and flexion/extension are performed at independent orthogonal joints, it is surprising to see some muscles at one joint being modulated with loads at the other joint. These results show how some muscles are controlled differently at the elbow and wrist. This coupling is perhaps important for counteracting inappropriate torques produced by multi-joint muscles that are necessary for torque production, such as elbow muscles which generate flexion torques by the biceps which generates elbow flexion torques as it aids in supination.

This work is supported by NIH grant NS-19331.

The role of the cerebellum in the generation of goal-directed, single joint movements was studied in rats with cerebellar deficits. Step-tracking elbow movements were performed at 4 amplitudes and under 3 instructions. Peak movement duration (MP) increased linearly with amplitude. Mean MP and MD values showed slight differences between cerebellar and control groups. Variability of MP and MD, however, was greater in the cerebellar group, especially during slow movements. Marked differences in the relative durations of acceleration and deceleration (symmetry) of the velocity profile occurred across all movement conditions. In all patients, both continuous and discontinuous velocity profiles showed periodical decelerations. The degree and variability of movement asymmetry increased as the demand for accuracy increased.

These findings support the thesis that movement trajectory, particularly the deceleratory phase, is under cerebellar control.

(Supported by a von Humboldt Fellowship to SHB)


Anatomical and electrophysiological evidence suggests a role of the red nucleus (RN) in limb movement, yet its importance is still not understood. The early work of Lawrence and Kuyper found that sectioning of the rubrospinal tract produced deficits in the use of the ipsilateral arm, whereas combined sectioning of the rubrospinal and climbing fibers produced transient deficits in the preferred paw to assess the accuracy of fast and slow movements with visual guidance (screen cursor visible) and without visual guidance (screen cursor blinded). In addition, patients had to examine the time required to adjust the direction of visually guided movements when the tablet was rotated 45°. In deafferented patients, rapidly visually guided movements from one target to another could only be delayed by fragmentation of the trajectory into smaller segments with lower than normal peak velocities. When vision was prevented, patients showed errors in amplitude and direction that were markedly greater than those of normals. Whereas, in the absence of visual feedback, normals could reproduce a given trajectory several times without substantial changes, in patients, the accuracy of sequential movements was further degraded with each repetition. In contrast, one patient thus far tested did not show any specific impairment in the ability to adapt to rotations of the relationship between the spatial coordinates of the hand and of the screen cursor. We conclude that somatosensory information from the limbs is necessary to accurately specify the direction and amplitude of responses aimed to visual targets, but may not be necessary to learn a visuomotor transformation.


Problems in motor control are difficult to address with a quantitative model of the motor plant. To assist experimental investigation of locomotor and voluminal formlimb movements in the cat, a preliminary computer model of its skeletomuscular system has been constructed and applied to an analysis of the step cycle. The approach is based on the calculation and experimental establishment of coordinate frames that are intrinsic to the skeletal-muscle-mechano-sensory system. The model is used to calculate intrinsic coordinates from a graphic representation of joints and skeletal muscle attachments was adopted from models of head movements in cats. (Pellizzoni and Peterson, in: Control of Head Movements, Oxford U. Press).

Specifically, the pulling directions of ten forelimb muscles were determined by first assessing the origin and insertion of each by dissection and then calculating the direction the limb moves from specific locations in the step cycle when the muscle is shortened. These pulling directions were checked qualitatively by observing the forelimb movements evoked by sustained stimulation of each muscle. Next these directions were used to establish the axes of an intrinsic coordinate system on which the model was based. The most striking finding was that the spatial features of the trajectories could be predicted based on the intrinsic coordinates and the direction of the intention vectors, revealing the functional geometry underlying the execution of the locomotor cycle. Predicted and observed trajectories were compared to determine the extent to which the intrinsic coordinate system has to be updated to yield a movement whose trajectory was similar to that found experimentally. This updating required the recalibration of the Eigen vectors and the components of the moment tensor during movements. The application of this approach resulted in the generation of a curved geodesics) or trajectories which approximated the locomotor behavior of the animal. These data provide a rationale for further pursuing the basis for the functional geometry underlying the spatial properties of limb movements.

(Supported by NIH grants NS29299 and NS21928)


Anatomical and electrophysiological evidence suggests a role of the red nucleus (RN) in limb movement, yet its importance is still not understood. The early work of Lawrence and Kuyper found that sectioning of the rubrospinal tract produced deficits in the use of the ipsilateral arm, whereas combined sectioning of the rubrospinal and climbing fibers produced transient deficits.

Rats were trained to reach for food through bars separated by 9 mm, and their paw preferences were established. The RN contralateral to a ball and to their preferred paw was then infused with ibotenic acid, and their non-preferred paw was bandaged to prevent use. The number of times the rats successfully reached for food with its preferred paw was compared to pre-operative success rates.

Histology showed complete cell loss in both the parvicellular and the parvocellular divisions of RN, with minimal damage to nearby structures. Such lesions seem not to impair reaching. These results support previous notions of partially redundant pyramidal and rubrospinal tract function.

385.4 EFFECTS OF GLOBUS PALIDUS LESIONS IN RATS ON BASSILIC FOREARM FORCE ENFORCEMENT. J.W. Harrell and B.C. Hides*, Dept. of Psychology, Hampden-Sydney Coll., Hampden-Sydney, VA 23843

Rats were trained to press with their forepaws on a force transducer under a fixed-ratio 5 (FR5) schedule of reinforcement for water reinforcers (Harrell & Hayes, Neurosci. Abst., 16:1224, 1985). Four rats received bilateral, electrolytic lesions of the globus pallidus (GP) and two rats were unoperated controls.

Rats were trained on the required task until a stable baseline of 20 days data was collected. Surgeries were then performed following: day testing under preoperative conditions. The initial postoperative testing period lasted 27 days. Thereafter ensued a six week hiatus followed by 57 additional testing days. A total of 100 days elapsed from performance of surgery to the conclusion of testing.

Three of the four GP rats showed relatively permanent effects of the lesioning. These effects, shown in strip chart recordings, were characterized by a decrease in ability to perform the rapid bursts of responses and an increase in the time between bursts. There were also noticeable changes in postural adjustment capability. While greater performance deficits were observed in this study than in previous studies of caudate nucleus lesions in rats on the same task, essential ability to perform the forelimb response remained unaffected.

(Supported in part by a grant from the Oswathmy Foundation of Virginia and an H-SC student research award)
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT VII
THURSDAY AM

pathway in fine finger movements. A task was designed to assess the
ability of subhuman primates to perform, fractionated (individual), ballistic
and temporal properties of linkages between RNm and limb muscles. A major
criteria for distinguishing between causal and non-causal linkages. Here we present
ATE CONCURRENTLY. DOPAMINE ANTAGONIST ALTERS THE TRAJECTORY OF
finger movements that required complex multiarticulations (flexion-
extension combinations). A second task was designed which assessed
the present experiments examined the role of the dorsal column (DC)
pathway in fine finger movements. A task was designed to assess the
ability of subhuman primates to perform, fractionated (individual), ballistic
finger movements that required complex multiarticulations (flexion-
extension combinations). A second task was designed which assessed
combining several tasks with significantly different temporal sequences of muscle
activation reduces non-causal correlations toward zero, without affecting correlations in
casual linkages. Experimental data supporting this conclusion was obtained from
monkeys trained to perform several movement tasks, each of which evoked distinctly
different EMG patterns. RNm unit discharge was recorded simultaneously with EMG
activity from 8-14 flexor muscles. We assessed the extent of behavioral coupling
between muscles with cross-correlations between EMGs. When behavioral coupling
was decreased, the number of muscles that remained well correlated with RNm
discharge also decreased. Correlations that remain under these conditions may represent
causal linkages, whereas those that disappear are likely to be non-causal.

385.7
Finger movement disorders following section of the dorsal columns.
385.8
FIELD OBSERVATIONS OF HAND MOVEMENTS AFTER DORSAL COLUMN
AND THE MOVEMENTS DIFFERENTIALLY CONTROLLED BY EACH. S.A. Kane, J.W.
FASTIGIAL, INTERPOSED, AND DENTATE NUCLEI: SOMATOTOPIC ORGANIZATION
of the monkey. (Supported by NS 13516 and NS 17474.)

385.5
TASK DEPENDENCE OF CROSS-CORRELATIONS BETWEEN
MONKEY RED NUCLEUS AND FORELIMB MUSCLE EMG.
L.E. Miller, P.L. van Kant, and J.C. Hauk.

385.10
REACHING TO FAR VS. NEAR CONTRALATERAL EXTRACORPOREAL
HEMISPACE BY MONKEYS WITH HEMINEGLECT FROM CORTICAL
LESIONS. Barbara K. Deuel. Department of Pediatrics, Washington
Univ. Sch. of Med., St. Louis, MO 63110.
In monkeys with cortical removals leading to neglect of
corporal and extracorporeal space, ipsilateral (IPSI) hand use is often impaired in contralateral (CONTRA)
spaces. To test the latter a salient bit of bait near (15°) or far (70°) from visual fixation, the animal was seated in a primate chair with its head in a viewing box and the hand (or furthest lesion) restrained. When the head was voluntarily fixed forward and the IPSI hand was voluntarily resting on the chair back, the viewing box was rotated 90°. If the eyes were fixed forward, a piece of bait appeared in one of four
quadrant. The latency from bait presentation to breaking of a light beam by the animal
was measured. Preoperatively, in the 15° reach most IPSI hands were faster in the IPSI hand than in the CONTRA hand, and the CONTRA hand tended to shorter latencies in the CONTRA field, with cross reaching adopted as an efficient strategy. Postoperatively animals tended to reach latencies than preoperatively in both 15° and 70° IPSI fields, contrasted with increased latencies in the CONTRA fields; however, without gradation of severity from 20° to 15° CONTRA fields for the group. Individual choice of motor strategy rather than an obligatory visual neglect of far contralateral space appears to have determined these results.

385.9
DOPAMINE ANTAGONIST ALTERS THE TRAJECTORY OF THE ELBOW JOIN BUT HAS NO EFFECT ON THE FINAL
POSITION GENERATED BY MONKEYS POINTING AT VISUAL TARGETS. R.M. Wyle and A. Martinez-Arizala.
Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20370.
To better understand the role of the nigro-striatal system in movement, we have studied the effect of a dopamine antagonist, metoclopramide, on an elbow flexion task performed by Rhesus monkeys. Three monkeys were trained to track a visual target by rotating their forearms about the elbow in a horizontal plane. In one animal, the target moved stepwise and the monkeys were required to point to it within five degrees of it. Successful responses were rewarded with a liquid nutrient. Unsuccessful trials earned decreased acceleration, velocity and position. Effective doses (0.3 to 0.4 mg/kg iv) reduced the effect of acceleration, velocity and position, but had no effect on final position, which was set by the use of the flexor and extension compartments. This suggests that the system compensates for the reduction in acceleration. We see evidence of compensation in the increase in the rise time of acceleration effects. The increase in the duration of the period of positive velocity, that period during which the arm moves toward the target. We cannot resolve the issue whether this effect is related to normal changes or reflects the operation of a feedback control system.

385.6
FIELD OBSERVATIONS OF HAND MOVEMENTS AFTER DORSAL COLUMN
SECTION. C.M. Leonard, C.J. Vierck, Jr., and B.Y.
Cooper, Dept. Neuroscience, Box J244, JHMHC, University of Florida, Gainesville, FL 32610.
The accompanying abstract by Cooper et al. reports that
monkeys (Macaca arctoides) with dorsal column lesions are
permanently impaired in performing multi-articulated,
fractonated fine finger movements in an experimental paradigm. This permanent deficit contrasts
with a temporary decrease in the use of the effect for limb
walking, climbing, and support generation. It was observed that monkeys showed grossly normal gross
movements within 3 weeks of surgery, including the ability to precisely oppose finger
and thumb in the precision grip. The 4th animal demonstrated apoposis at 3 months. Frame by frame
comparisons of pre- and post-surgery videotapes reveal occasional subtle changes in the spacing between the digits that suggest altered mechanisms of control. There is a long-lasting decrease in the use of the limb
for self-scratching, foraging and manual sexual investiga-
tion. The decrease in scratching was unexpected. Scratching is a stereotyped behavior performed at a
fixed rate of 5/sec and does not require either multi-
articulation or individual finger movements. It had not
previously been thought to depend on intact dorsal
columns. Field observations conducted with specialized
laboratory tests can provide new insights into dorsal
column function. (Supported by NS 13156 and NS 17474.)

385.11
PREOPERATIVE WESTERN MONKEY WITH HEMINEGLECT FROM CORTICAL
Lesions showed grossly normal function in the movements
seen in the present study, but deficits were noted in the
motor movements to the ipsilateral side. The deficits included:
1) a decrease in the fine movements of the hand,
2) a decrease in the fine movements of the head,
3) a decrease in the fine movements of the trunk,
4) a decrease in the fine movements of the legs,
5) failure to track a moving tactile cue (fine
grasping) is a stereotypical behavior performed at a
unified rate of 5/s and does not require either multi-
ariculation or individual finger movements. It had not
previously been thought to depend on intact dorsal
columns. Field observations conducted with specialized
laboratory tests can provide new insights into dorsal
column function. (Supported by NS 13156 and NS 17474.)


Utility of a multidimensional approach is twofold. First, quantitative interpretation of experimental results for suitable comprehensive handling (cf. G. J. G. Nispel, J. Pellionisz, & E. D. Ukena, 1986). Second, in order to implement, in neurocomputing and (near)robotics, a theoretical understanding of how biological organisms coordinate movements, a formal expression of the underlying mathematical principles is necessary, such as coordination in Eigen-vector-space (cf. Pellionisz, 1984). The present preliminary model (representing 2D movements) of the human forearm is based on anatomical data, and is aimed at interpreting physiological measurements, both also obtained in the laboratory. A video will demonstrate the present level of the suitability of the model to geometric CNS motor control in general non-orthogonal intrinsic coordinate frames and their underlying characteristic Eigen-vector-systems. Supported by NS-22999.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM III

THURSDAY AM

ACTIVITY AND PONTO-BULBAR CONNECTIONS OF RETICULO-SPIRAL NEURONS MONITORED ELECTRICALY IN CONTINUOUSLY RECORDING HEAD MOVEMENTS. A. Graftyn, O. Hardy, and A. Berthoz.

Lab. de Physiologie Neurosensorielle, CNRS, Paris, France. Descending axons of rostral medulla were recorded intracellularly in alert, head-fixed cats performing orienting to moving visual stimuli. Spike activity was examined for correlations with eye movements and disynaptic excitation from the contralateral superior colliculus were recorded intracellularly in alert, head-fixed cats trained to perform saccadic and smooth eye movements. The rostral SC contains an extensive representation of the visual field. Here we report the discharge characteristics of TRSNs located in the central portion of the colliculus. Antidromically identified TRSNs were recorded extracellularly in head-fixed/free cats trained to perform several visual-motor tasks. Activations revealed a continuum which can be illustrated by 3 representative groups: 1) Phasic RSN generating short bursts linked to the onset of saccades; 2) tonic saccade-related component; 3) RSN showing prolonged bursts, frequency modulated in relation to saccades and early parts of dynamic EMG-components. 1) with modulation of spike rate reflecting the profile of even the longest EMG-transients and nearly lacking saccade-related components. HRP labeling reveals that RSN of groups 1, 3) contact the reticulospinal core only, by a few (1-5) poorly branched collaterals. Group 2 RSN show multiple (8-15 collaterals) branching in the ponto-bulbar RF, prepositus/vestibular complex, and abducens and facial nuclei. The data indicate a distributed nature of the premotosor motor neurons in the tecto-reticulospinal network and disclose some aspects of its intrinsic connectivity.

HEAD MOVEMENTS. A. Grantyn, O. Hardy and A. Berthoz.

NEURONS SUBSERVING VISUALLY TRIGGERED ORIENTING EYE MOVEMENTS AND PONTO-BULBAR CONNECTIONS OF RETICULO-SPINAL NEURONS. K. Kawano, Y. Watanabe, and S. Yamane. Bionics Section, Department of Electronics, Kyushu University, Fukuoka, Japan. The mesencephalic reticular formation of Macaca fascicularis monkeys was systematically explored for evidence of an eye movement signal which could be used to position or transform information through single unit recording, microstimulation and microinjection of muscimol. Eye rotations in 3D were recorded. Constant frequency microstimulation in the vicinity of the nucleus of the central tegmental tract (C) induced constant velocity eye rotations which held their position when stimulation ceased. Rotations were clockwise for stimulations on the right side and counterclockwise on the left with variable vertical components. As reported elsewhere (Hepp et al. 1986), unilateral injections of muscimol in the rMLF produced tonic torsional deviations of the eyes. These tonic deviations became leaky with unilateral injections between the rMLF and INC, producing clypeus neurons (OPNs) that initiated or terminated saccades involved in saccade generation. We speculate that rostral TRSNs may provide excitation to OPNs, thereby suppressing orientation during attentive fixation.

ROSTRAL OUTPUT NEURONS OF SUPERIOR COLLICULUS ARE ACTIVE DURING ATTENTIVE FIXATION. B.P. Mancz and D. Guttman.

Neurology and Neurosurgery, McGill University, Montreal, Canada. The superior colliculus (SC) plays an important role in controlling orienting behaviour. A major projection neuron emanating from the deeper layers of the cat SC, the tecto-reticulo-spinal neuron (TRS), collaterizes in regions of the brainstem and spinal cord. To study the responses of rostral SC head and eye movements. The rostral SC contains an extensive representation of the visual field. Here we report the discharge characteristics of TRSNs located in the central portion of the colliculus. Antidromically identified TRSNs were recorded extracellularly in head-fixed/free cats trained to perform several visual-motor tasks. Activations revealed a continuum which can be illustrated by 3 representative groups: 1) Phasic RSN generating short bursts linked to the onset of saccades; 2) tonic saccade-related component; 3) RSN showing prolonged bursts, frequency modulated in relation to saccades and early parts of dynamic EMG-components. 1) with modulation of spike rate reflecting the profile of even the longest EMG-transients and nearly lacking saccade-related components. HRP labeling reveals that RSN of groups 1, 3) contact the reticulospinal core only, by a few (1-5) poorly branched collaterals. Group 2 RSN show multiple (8-15 collaterals) branching in the ponto-bulbar RF, prepositus/vestibular complex, and abducens and facial nuclei. The data indicate a distributed nature of the premotosor motor neurons in the tecto-reticulospinal network and disclose some aspects of its intrinsic connectivity.

NEURONAL ACTIVITY IN THE POSTERIOR PARITAL CORTEX (AREA PG) OF ALERT MONKEYS DURING OCULAR FUSION RESPONSES. K. Kawano, Y. Watanabe, and S. Yamane. Bionics Section, Electrotechnical Lab., Tsukuba-shi, Ibaraki 305, JAPAN. Previous studies showed that a group of neurons in the posterior parietal cortex (area PG) of the monkey are activated by movements of the entire visual field. In this study, data were collected concerning their relation to the ocular following responses, which are always elicited by movements of the visual field in alert behaving monkey. A monkey faced a screen onto which a random dot pattern was projected, and its eye movements were recorded with the magnetic search coil technique. Single unit activity was recorded in the posterior part of area PG. 166 neurons were activated by the movements of the visual scene showing directional selectivity. Most of the neurons showed similar dependence on the visual properties of the stimulus to that of ocular following, e.g., preference to high speed (80-160°/s), latency delay due to blurring and oscillation at the high temporal frequency ranges were very short. Some of the neurons were activated less than 50ms after the onset of the stimulus, and about half of them started their increase of firing rate more than 10s before the eye movement. Furthermore, some of them were activated antidromically from the ipsilateral cerebral peduncle. These results suggest an important role of the neurons in the posterior part of area PG in the initiation and control of ocular following.

SMOOTH EYE MOVEMENTS ELICITED BY MICROSTIMULATION IN THE FRONTAL EYE FIELDS REGION OF ALERT MONKEYS. M.G. MacAvoy, C.J. Bruce, and J. Guitenti. Sec. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510. Smooth eye movements were studied in conjunction with microstimulation of the frontal eye fields (FEF) of rhesus monkeys. Eye movements were recorded with a search coil in one eye. Both eye position and eye velocity were digitized for later analysis. Our primary observation is that smooth eye movements, rather than saccadic, were elicited by microstimulation of the posterior region of the motor cortex. Smooth movements were seen using currents as low as 10-50µA during both anteropropulsion and spontaneous ocular motor behavior. These eye movements usually had a duration of 250-500 ms. Eye velocities up to 25°/sec were obtained, with velocity increasing as a function of current. Each eye had a characteristic direction of movement. Vertical, horizontal, and oblique movements were seen. The horizontal component was almost always ipsilateral to the stimulating electrode, in contrast to the contralateral saccades usually obtained with microstimulation of the nearby saccadic FEF. The region over which smooth eye movements were elicited is near the ventral (inferior) FEF region and oculomotor cortex. Smooth eye movements were elicited with a latency delay due to the propagation of the current. Injections in the INC produced either no variable change in set point with both vertical and horizontal movements, or only a vertical drift or a variable change in set point with both vertical and horizontal movements. Goal directed saccades did not overshoot as expected with a loss of position feedback. The results suggest that the velocity to position transformation involves the INC and that the position signal is not used to terminate saccades.

SMOOTH EYE MOVEMENTS ELICITED BY MICROSTIMULATION IN THE FRONTAL EYE FIELDS REGION OF ALERT MONKEYS. M.G. MacAvoy, C.J. Bruce, and J. Guitenti. Sec. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

SMOOTH EYE MOVEMENTS ELICITED BY MICROSTIMULATION IN THE FRONTAL EYE FIELDS REGION OF ALERT MONKEYS. M.G. MacAvoy, C.J. Bruce, and J. Guitenti. Sec. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

SMOOTH EYE MOVEMENTS ELICITED BY MICROSTIMULATION IN THE FRONTAL EYE FIELDS REGION OF ALERT MONKEYS. M.G. MacAvoy, C.J. Bruce, and J. Guitenti. Sec. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

ELECTRICALLY EVOKED TURNING: ASYMMETRIC AND SYMMETRIC COLLISION BETWEEN ANTEROMEDIAL CORTEX AND STRIATUM. J.S. Yeomans and P. Ruckenbah. Psychology, U. Toronto, Canada. Stimulation of anteromedial cortex (AMC) resulted in contraversive circling mixed with ipsiversive circling in one rat. Stimulation of striatum resulted in smooth contraversive circling. In the collision test, at low currents collision only was 15% but at high currents collision was near 40%. In both cases, summation at long C-T intervals was only 20%. Collision occurred, it seems, in the corticostriatal interneuronal connections mediating circling, but not in cortical outputs mediating ipsiversive circling. The collision was asymmetric at both low and high currents. That is the response was rapid (1.0 ms) when the C pulses were delivered to the striatum and the T pulse was delivered to AMC, but the recovery was much slower (0.6 to 1.0 ms) when the C pulses were delivered to AMC and the T pulses to the striatum. The rapid recovery (0.6 to 1.0 ms) was symmetric 20% collision, asymmetric 80% collision. The slow recovery (1 to 4 ms) was asymmetric. The asymmetric response is proposed to be due to activation of cortical interneurons, resulting in the appearance of longer latencies than are delayed by 2 ms. The asymmetry results from the inability of transynaptic action potentials to cross the synaptic barriers. The refractory period curve in AMC rose at the same long C-T intervals (2-4 ms), perhaps also due to blockade of transsynaptic action potentials.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM III


The "colliding saccade paradigm" (microstimulation applied during an ongoing saccade) differentiates brain sites processing (a)retinal error vs. (b) motor error. If (a) is the case, the trajectory of evoked saccades optimizes for the intended displacement. If (b) is the case, the saccade is accurately directed towards the target. This implies that the compensatory evoked saccades are directed toward a goal defined by eye position. The time at which eye position is sampled was studied in monkeys by varying the interval between initial saccade and trajectory correction testing to 2 sec or 0.5 sec. In two experiments: (1) the artificially created retinal error is referred to an eye position synchronous with the presumed real target onset (i.e. the original stimulus), in order to compute spatial error and motor error (Robinson, 75); (2) the artificial retinal error is referred to the synchronous eye position (e.g. Jurgens et al., 81).

Compensation for changes of eye position during saccade as well as saccade latency varies with target eccentricity, but it is not clear whether this relationship is based on the craniotopic or retinotopic coordinates of the target. Instead of testing only saccades made from primary gaze, which keeps target eccentricity with respect to eye and head identical, we tested saccades made from both primary and eccentric gaze positions. We measured saccades by electrooculography in 13 normal subjects. Each was instructed to make prompt, accurate saccades to a 3.5° target light that stepped unpredictably to 1 of 7 positions between ±10° and ±30°. Subjects performed 450-900 saccades in one 60-90 minute session. We performed multiple linear regression analysis of rightward and leftward saccades combined. Latency covaried significantly with target step size (R² = 0.58, p < 0.001) but not with initial and final target position. These results suggest that the effect of target eccentricity on saccade latency may reflect retinotopic coding of target location. (Supported by the Rock Foundation and NIH-EY-07047)

PIERSON'S LAW FOR SACCADIC. P. Doma and P.E. Balliet (SPON: L.C. Doering), Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Saccadic latencies were measured to small light stimuli in darkness for two different tasks—foveating or anti-saccades. The stimulus is the target for a foveating saccade, or is the cue for an anti-saccade which peripheralizes the retinal image of the cue. Stimulus luminance, wavelength and retinal adaptation were varied. For either task Pierson's law fits pure rod or pure cone data with an exponent of -1.00, not -0.33 as others suggest. (a) For an exponent of -1.00, Pierson's law reduces to an ocularmotor version of Bloch's law of temporal integration; latency is the sum of a minimal transit time from the photoreceptors to the eye muscles, plus a variable waiting or "response time" for a threshold number of photons, (b) This number for the rods is comparable to the classical perceptual limit of about 100 photons. The corresponding number for cones is roughly a factor of 5 higher than the cone threshold for perception. The implication is that rod-driven saccades are more perceptual in nature in comparison to the more reflex cone-driven saccades. (c) For foveating saccades latencies for mixed rod and cone inputs are substantially shorter than for the rods or cones alone—this is a synergistic effect. For anti-saccades the same stimuli show no synergism. A neurophysiological mechanism can be proposed.
Animals must be able to orient effectively to nearly simultaneous stimuli of different sensory modalities. We investigated the effects of inter-stimulus delay on saccadic orientation responses of cats trained by an autoshaping paradigm and presented with two disparate targets of auditory, visual, or mixed modality. Saccadic latency increased, perhaps reflecting need for a complex decision, whereas reaction time (a different modality) were simultaneously presented and a single saccade to an intermediate location resulted. If one stimulus preceded the other by less than about 100 msec, a single saccade to the target or to an intermediate location resulted, indicating that decision (choice of one of the two targets) or summation occurred. At intervals < 100 msec, an initial saccade toward one location, followed by another, formed an inter-saccadic mid-stream, a saccade to a location intermediate between the two targets. For intervals > 250 msec, two sequential saccades toward the two stimulus locations often resulted, reflecting the order of stimulus presentation.

Multisensory enhancement or depression manifested by neuronal responses in optic tectum, a center for sensory motor integration for orienting behavior, can be strongly influenced by the relative timing of the stimuli. There may be complex spatiotemporal integration of teat point images; selection of one vs integration of two competing stimuli may result from such neural interactions.

**MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM IV**

**387.1**

**ORBITAL GEOMETRY OF THE ACCESSORY LATERAL RECTUS MUSCLE IN MACAQUE MONKEYS, R. G. Booth, M. V. Joosse* and W. W. Quick. Yerkes Primate Research Center, Depts. of Physiology and Ophthalmology, Emory Univ., Atlanta, GA 30322.**

Simmans have an extraocular muscle, the accessory lateral rectus, that is not present in either great apes or humans. Its functional role in monkey eye movements is usually considered to be minor due to the fact that it is short, weak, and attaches far back on the globe (Fuchs & Luscher, J. Physiol., 1971). However, no quantitative measurements or predictions of its function are available. Recently, Miller and Robins (Vis. Res., 1987) have applied a model of the effects of muscle force on human eye movements ("SQUINT") to monkeys. Input parameters for this model are based on quantitative dissections of positions of origins and insertions, and measurements of lengths and cross-sectional areas of each extraocular muscle. Values of these parameters for the accessory lateral rectus are unknown. In order to fill in this gap and provide a basis for making quantitative predictions about the functional role of this muscle, we have made measurements of these parameters for 10 eyes of 5 monkey cadavers (4 Macaca mulatta and 1 Macaca nemestrina). Mean values (expressed of origins and insertions, and measurements of lengths and cross-sectional area = 3.56 sq mm).

**387.2**

**EXTRACULAR MUSCLE ROTATION AXES: DETERMINATION IN THE INTACT MONKEY BY MAGNETIC RESONANCE IMAGING. R. L. Villette*, S. J. Karlik* and T. Vilis. (spon. W. F. Brown) Depts. of Physiology and Diagnostic Imaging, University of Western Ontario, Sarnia, Ontario, Canada.**

As suggested by Simpson et al. (1986), contemporary data on the rotation axes of the extraocular muscles are necessary for current models of oculomotor control. The advantage of Magnetic Resonance Imaging (MRI) over dissection data is that muscle axes can be determined while the subject fixates a target straight ahead, thus giving normal innervations to each muscle. MRI scans of both orbits were carried out on 2 adult males with no strabismus. The scanner had a 1.5 Tesla magnet which gave a resolution of approximately 0.5 mm. Rotation axes, calculated using the centre of the eye and the centres of muscle cross sections, were rotated so that both medial recti were as near vertical as possible. Average direction cosines of the rotation axes are given (left eye). The reference axes are X (anterior positive), Y (left positive) and Z (superior positive).

**387.3**


If eye positions are described in terms of their rotational displacement from the normal straight ahead position, using an angular position vector which lies along the axis of the rotation and whose length is the size of the rotation, then all these vectors lie in a single plane -- a result known as Listing's law. We have used the magnetic field/search coil technique to measure the angular position vectors. When instructed to look around the room, with shoulders still, 5 of the subjects showed head positions restricted to 5° of a single plane -- a scatter comparable to what we have found for eye positions in humans and monkeys. The sixth subject, when looking above head level and another below head level. When subjects made repetitive eye-head gaze shifts between targets at 70° eccentricity, head positions remained roughly in the plane. Small systematic deviations were attributed to motion at lower cervical joints, because they were reproduced by voluntary forward or backward bending of the neck. The existence of Listing's laws for both head and eyes shows that the law is not a consequence of muscle geometry.

**387.4**

**HUMAN VERTICAL OPTOKINETIC NYSTAGMUS (VOKN): UP-DOWN ASYMMETRY WITH AND WITHOUT CENTRAL RETINAL STIMULATION. G. M. Muraszko* and J. P. Howard (spon. J. R. Mendeleson, Dept. of Psychology, York University, Toronto, Ontario, Canada.**

In cats and monkeys the slow-phase gain of VOKN is higher for upward than for downward stimulus motion. The presence of a consistent VOKN asymmetry in humans is controversial, probably due to use of electrooculography which produces an eyelid artifact. We used the magnetic search coil method to measure the monocular VOKN of 10 head-upright normal subjects. A 64° x 61° wide-random-dot display moved at velocities ranging from 10 to 70°/s. For seven subjects slow-phase gains were 40% higher in the upward direction for velocities at and above 30°/s. Three other subjects showed symmetrical VOKN. When a vertical band 6° wide spanned the center of the moving display, two patterns of results emerged. In six subjects, including one who showed a symmetrical full-field velocity, central occlusion caused a larger drop in downward VOKN than in upward VOKN for stimulus velocities of 50 and 70°/s. The other four subjects showed equally poor (<10%) VOKN in both directions. They were re-tested with a narrower occluder (3°) with little increase in evoked eye velocity. Thus, an upward dominance in VOKN is prevalent in humans at higher stimulus velocities, and the slower downward response is relatively more susceptible to the effects of central retinal occlusion. Investigations of response properties of neurons in the cat's accessory optic system have revealed that the visual cortex is a major source of upward direction-selectivity and high-velocity tuning, whereas downward direction-selective cells receive their input directly from the contralateral retina (Grasse, K. L. et al., Exp. Brain Res., 55: 69, 1984). Our results suggest that human VOKN may involve similar pathways, and at higher stimulus velocities the cortically mediated upward VOKN is more vigorous and more resistant to occlusion of the central retina than the directly innervated downward VOKN.

Subjects were presented with a sinusoidally moving optokinetic inducing field (1/4 Hz). Target retinal feedback was varied from 0 (target foveally at all times) to 1 (-1) (target foveally at all times). With 0 feedback there was substantial suppression of OKN; however, moderate amplitude slow eye movements remained, counteracting the sinusoidal target. With 1 feedback, target velocity suppression appears to come from target-field relative motion and attention—via Rea ‘84, Soc Neurosci ‘87). As feedback increased, amplitude and phase of this residual movement decreased until with 1 feedback there was very little movement. The decrease was particularly rapid for small amounts of feedback presented near the stabilized condition. Linear models cannot account well for this decrease in amplitude and phase. However, a simple model with a non-linearity at its input predicts the results nicely. The non-linearity has a gain which is high near the fovea, but decreases with eccentricity from the fovea.

These findings indicate that suppression of OKN comes largely from the VOR, with retinal slip serving to fine-tune the suppression. The model suggests the existence of a fixation mechanism whose sensitivity is maximum at the fovea. (Supported by NSF BNS-85-19267)

387.7 COMPARISON OF VESTIBULO-OCULAR REFLEX (VOR) MODIFICATION METHODS IN CATS. W. Freedman, J.R. Carroll*, and J.G. McElgin. Dept. of Electrical and Computer Engineering, Phila, PA 19104, Pennsylvania College of Optometry, Phila, PA, Temple University School of Medicine, Dept. of Pharmacology, Phila, PA 19140, and Moss Rehabilitation Hosptial, Phila, PA.

The vestibulo-ocular reflex (VOR) has been measured and optically modified in several animal species. VOR gain can be increased optokinetically by placing a head-fixed target on an optokinetic drum and sinusoidally oscillating it in a direction opposite to that of the drum rotation. VOR increase can also be accomplished by having an animal wear a set of magnifying lenses. We describe here a comparison of three methods for producing VOR increases in cats using an (i) optokinetic drum, (ii) a pair of 2.2X telescopic lenses, or (iii) a Fresnel lens (goggles that we have recently developed in our lab). After initial calibrations to test the VOR in the dark and the light, VOR modification periods of 15 minutes were each followed by a 1 minute period to test the VOR. The results of the comparison in 4 cats show that the Fresnel lens system produces a greater and more stable VOR gain increase than the other two systems. After 2.5 hours of modification, the VOR gain increases (n=4) were optokinetic drum-1.15, 2.2X telescopes-1.25; Fresnel lens-1.40. These results show that some animals that had poor or minimal VOR modification, now exhibit more robust VOR gain increases using the Fresnel lens system. (Supported by a grant from NSF BNS-8410231) and NIH (507-RR05417)


The temporal characteristics of the central systems used to cancel the vestibulo-ocular reflex (VOR) were studied in squirrel monkeys who were trained to fixate a target that moved with the head. Monkeys were passively rotated on a vestibular turntable in the horizontal plane. Two types of experiments were performed, one at a constant velocity of 20/sec during VOR cancellation, and was then rapidly accelerated to a new velocity of either 50/sec or -10/sec. This unpredicted change in head velocity produced a transient change in the opposite direction. The transient had a duration of 140-210 msec, after which the eye velocity returned to zero. The gain of the VOR during the transient response was less than one, in respect to the step change in head velocity, after a delay of 10-15 msec. 2) A second series of experiments were designed to determine the frequency response characteristics of the cancellation system. The monkeys were rotated sinusoidally at a low frequency (0.1-0.9 Hz), and once eye velocity was cancelled, the frequency of rotation was increased to 1.0-6.0 Hz. VOR gain was zero for stimulus frequencies below 1.2 Hz during rotation, but was in phase for stimulus frequencies above 4.0 Hz. Thus, the failure of the cancellation system to completely cancel the VOR during unexpected changes in head velocity probably reflects the fact that the input to the cancellation system is only a low pass filtered copy of the signals that drive the VOR.

387.10 HUMAN SUBJECTS SUPPRESS THE VESTIBULO-OCULAR REFLEX DURING VISUAL PURSUIT. J. Masuoka and B.W. Freeman. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

We have shown previously that when moving subjects suppressed their moving target’s maximum smooth eye velocity is less than the sum of their VOR and smooth pursuit responses when measured independently. We now report that this is because the suppression of pursuit occurs when pursuing moving targets. This occurs when the VOR is dominated by pursuit. The latency of the VOR (onset of head rotation to onset of eye-in-orbit rotation) was 12 msec, which is shorter than values reported with lower visual stimuli in man. Peak gaze velocity (group mean) was 30 deg/sec, which was 45% of peak head velocity, and still occurred during each head perturbation. The mean predominant frequency of head rotations was 11 Hz. These results are relevant to the performance of the VOR during running, when head rotations of similar amplitude and acceleration may occur. (Supported by NIH grant EY06717, NASA contract 9-17439 and the Veterans Administration)


The positions of each eye of the awake turtle, predefined by a small recording. Horizontal OKN was strongly asymmetric and non-conjugate during monocular stimuli, with a strong response by the exposed eye to stimulus moving in the nasal field of the other eye. Exposed eye contralateral to the occluded eye hardly moved as the exposed eye viewed nasal stimuli, but was more yoked during temporal stimuli. These dramatic differences between the eyes was also often observed during binocular viewing of identical random-dot patterns drifting horizontally, so that the slow phase velocity of the eye viewing the nasal stimulus was greatly smaller than the other eye. This non-conjugate OKN is clearest to stimuli of about 10 deg/sec. Concurrent with the non-conjugate slow phases was the yoked nature of the timing of fast phase eye movements.

OKN was also analyzed simultaneously for both eyes following monocular intravital drug injections. Bicuculline, known to evoke spontaneous temporal-to-nasal nystagmus in mammals with yoked eyes, elicited nystagmus mainly in the injected eye of the turtle. Likewise, monocular injections of APB, known to eliminate OKN in rabbits, exclusively blocks OKN of the injected eye, without affecting the eye movements of the other eye. Therefore, monocular OKN and effects of monocular drugs indicate that oculomotor pathways in the turtle can remain functionally independent for each eye. Supported by USPHS EY05978 and MH39015.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM

THURSDAY AM

AND INNERVATION OF PRIMATE LEVATOR PALPEBRAE SUPERIORIS

ANO INEXPENSIVE ULTRASONIC CONTACT LENS TO MEASURE ACCOMMODATION. K. Kozonasky and R. Remmel, Biomedical Engineering Dept., Boston University, Boston, MA 02215.

The levator palpebrae superioris (LPS) and orbicularis oculi (ObOc) are agonistic muscles functioning in blinks. Motorneurons innervating these muscles were identified in Cynomolgous monkeys by retrograde transport of HRP. LPS motoneurons were located bilaterally within the caudal central nucleus of the oculomotor nucleus. The lack of any apparent lateralization in motorneuron distribution and the high percentage of neurons labeled from injection of one LPS suggests that individual motorneurons may innervate both LPS muscles. Motorneurons innervating the ObOc muscle were distributed within the dorsolateral subdivision of the ipsilateral facial motor nucleus, with a few neurons in the corresponding locus of the contralateral nucleus.

Unlike the other extraculor muscles, the LPS lacks a layered distribution of fiber types. The ObOc, however, has the same 3 unusual multiply-innervated fiber types (SIF) found in the global layer of other extraculor muscles. Fiber types corresponding to extraculor muscle global red SIF (type 3) and global intermediate SIF (type 4), with only a few global pale SIF fibers (type 5) noted. The histochemical/morphological profiles of these fiber types are such that they do not respect "traditional" fiber classification schemes, but are consistent with a role for LPS in tonic elevation of the lid. The multiplex-innervated fiber types, which characterize eye muscles, were absent from the LPS fibers. These differences, together with physiological differences between LPS and ObOc reflect not only their distinct functional roles in blinking, but also their diverse embryological origins. NEI EY05464 (JDP) and EY07166 (PJM).

Pupil is being tested. The two crystals can be glued on with silicone rubber glue. A local anesthetic should be applied to the eye. A coil of wire can also be glued to the lens so that eye movements can be measured. Accummodation should thus be measurable for $300.

DRUGS OF ABUSE IV

DOSE RESPONSE EFFECTS OF COCAINE ON EVOKED ACTIVITY RECORDED FROM DORSAL RAPEPHALAMIC AND DORSAL PERVATICOMATIC AREAS. W. McVaugh, A. Shen* and N. Dafny (SPON: A. Schrommbr). Dept. Neurobiol. & Anatom., The Univ. of Texas Medical School at Houston, 77025.

Cocaine has been reported to have variable effects on CNS components. This study investigated the effects of cocaine on Evoked Responses (ERPs) recorded from Dorsal Raphe (DR) and Locus Ceruleus (LC) in the freely moving rat. In addition, the effects of Naloxone (an opiate antagonist) and Desipramine (a NE uptake blocker) were examined. Sixteen male Sprague-Dawley rats were permanently implanted with 120 mm stainless steel semimicro-electrodes. Postsynaptic Evoked Responses were recorded before and after the administration of saline, cocaine, Naloxone, and Desipramine. The ERPs were analyzed in the LC, the P3 amplitude increased with each increasing dose. P3 exhibited facilitation at 1 mg/kg, but was inhibited at higher doses. P3 from DR demonstrated a dose-dependent facilitation, while P3 was inhibited. Naloxone had no effect in either structure, while treatment with desipramine augmented the response from LC. In conclusion, it was seen that (1) cocaine affects various CNS sites differently in dose-dependent patterns; (2) cocain can differentially affect the individual components of the SER; and (3) cocaine's effects do not seem to be mediated via opiate receptors.


Neurotoxin lesions of discrete brain regions have been helpful in determining the involvement of specific neuronal systems in drug abuse. The neurotoxin 5,7-dihydroxytryptamin (5,7-DHT) which destroys neurons containing serotonin, increased amphetamine self-administration when injected intraventricularly. However, a localized lesion of the nucleus accumbens did not alter drug intake (Lyness et al., 12, 937-941). It was hypothesized that lesions in the nucleus accumbens would result in different effects of cocaine. The effects of 5,7-DHT lesions on cocaine drug discrimination was determined.

Adult male Fischer-344 rats were trained to discriminate saline (10 mg/kg, IP) from saline using a standard drug discrimination paradigm. Subjects were then tested using a cumulative dosing procedure. After the cocaine discrimination was acquired, generalization gradients were determined. Rats were then lesioned with the neurotoxin and generalization gradients were redetermined. The neurotoxin treatment resulted in attenuation of the discriminative stimulus properties of cocaine. Supported by USPHS Grant DA-03631.
388.3 TOXIC CONSEQUENCES OF COCAINE ARE AUGMENTED BY NONCORTICOSTEROIDAL INFLAMMATORY PROSTAGLANDINS. J. P. DaVanzo*, C. Volmer* and S.J. Dworkin*. Dept. of Psychiatry, LSU School of Medicine, Shreveport, LA 71130.

The pathological consequences of environmental events can depend on the functional relationships between behavior and the delivery of the event. Studies using behavioral models have shown that yoked animals are more likely to develop ulcers and other signs of physiological and neurobiological pathology compared to animals given the opportunity to postpone the stimulus. Additionally, the contingent delivery of intravenous morphine produced a greater suppression of the phrenic nerve turnover compared to noncontingent infusions.

Twelve littermate triads of male Fisher F-344 were prepared with chronic indwelling intravenous catheters and placed in individual operant conditioning chambers. Each of the 3 operant conditioning chambers were housed in individual sound-attenuating enclosures. Responses by the rat placed in the center of the enclosure resulted in the delivery of cocaine infusions (1.0 or 0.67 mg/kg) to both the center rat and his littermate on the right, while saline was infused to the animal on the left. Cokeine resulted in the death of only the rats receiving noncontingent injections within a relatively short time period.

Research Supported by NIDA Contract# 271-87-818

388.4 HEMODYNAMIC RESPONSE CHARACTERISTICS TO COCAINE (C) IN RATS. M. H. Kuepfer*, H. M. Wehner* and S.J. Dworkin*. Dep. of Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Our laboratory has reported that C produces small, dose-dependent increases in arterial pressure and mesenteric vascular resistance (MVR) and little change in hind-quarter resistance (HQR) in awake rats. We sought to describe the mechanisms by which the hemodynamic responses were mediated using receptor antagonists. Rats were instrumented with an arterial cannula for AP and heart rate (HR) determination and with miniaturized pulse Doppler flow probes for estimating changes in MVR and HQR. After recovery, C was administered alone or 10 minutes after pretreatment with selective antagonists. The table contains the results expressed as mean ± SEM (N’s = 4-12).

<table>
<thead>
<tr>
<th>DRUG (mg/kg)</th>
<th>AP</th>
<th>HR</th>
<th>HQR</th>
<th>MVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine (5)</td>
<td>14±3</td>
<td>35±13</td>
<td>3±18</td>
<td>44±30</td>
</tr>
<tr>
<td>Pentolinium (7.5)</td>
<td>12±3</td>
<td>13±6</td>
<td>13±6</td>
<td>17±6</td>
</tr>
<tr>
<td>Metyrapone (1)</td>
<td>4±2</td>
<td>2±7±5</td>
<td>2±0±13</td>
<td>1±2±4</td>
</tr>
<tr>
<td>Pramipexol (0.1)</td>
<td>-2±2</td>
<td>-3±6±4</td>
<td>-1±3±1</td>
<td>9±6</td>
</tr>
</tbody>
</table>

These data suggest that the most AP and HR responses are dependent upon α1-adrenergic receptors, that the HR response is due primarily to removal of central sympathetic tone (α1-adrenoceptors and those in the brainstem, often used clinically to treat C toxicity, actually exacerbates AP and HR responses to C. (Supported by HL37214, HL38299 and NIA, NIH, Affiliate.)


This experiment tested whether tolerance to cocaine was related to cocaine dependence/withdrawal. Animals trained to detect the discriminative stimulus properties of pentylenetetrazol (PTZ) can be used to assay for the occurrence of withdrawal from a variety of drugs of dependence, including cocaine. In addition to producing a PTZ-like stimulus, termination of high dose cocaine administration is also associated with tolerance to the discriminative stimulus properties of cocaine, suggesting that the occurrence of a PTZ-like stimulus during withdrawal may be a mechanism that produces the apparent tolerance to cocaine. To test this hypothesis, we trained one group of rats to detect the amnesic drug pentylenetetrazol (20 mg/kg), and a second group of rats to detect cocaine (10 mg/kg). Given acutely, 1) PTZ shifted the dose-effect curve for the detection of cocaine approximately 2-fold to the right; 2) diazepam did not antagonize or potentiate the cocaine cue, and 3) diazepam (5 mg/kg) antagonized the PTZ cue. Following these tests, cocaine-trained subjects were given cocaine, 20 mg/kg, every 8 hr for 7 days. Subsequently, the cocaine dose-effect curve was determined in various brain regions. The average time for the T > 0.10 mm from the occurrence of the cue was 23±6 min. Differences in the time to reach maximum brain concentration may account for differential biochemical effects in various brain areas.


The mood elevating and euphoric properties of cocaine coupled with its widespread abuse and highly addictive properties has stimulated research into the neurochemical basis of its behavioral effects. Recent evidence links the behavioral effects of cocaine to its interaction with the dopamine transporter. We recently synthesized [3H-N-11C-methy1]cocaine and measured its regional and temporal distribution in living (anesthetized, ketamine, isofluorane) baboon brain with high resolution PET. This was highest in subcortical structures bilaterally, peaking at 4 minutes and clearing to 30% of the highest value by 30 min. Ratios of striatum to cortex in baboons at peak uptake were 2.0 and 2.4 respectively. Plasma clearance of carbon-11 was rapid and at 30 min, more than 50 % of the radioactivity had been excreted as carbon dioxide. The high initial uptake of [11C]cocaine into the brain parallels the rapid mood elevation produced by the drug. The initial uptake of [11C]cocaine into the striatum suggests that the neurochemical mediator of behavioral activation may reside in subcortical structures. Research supported by SENE, ONH and NIH NS-15638.
EVIDENCE FOR A BIOPHASIC RESPONSE TO COCAINE IN RATS. L.E. Balster et al. Dept. of Pharmacology, Cornell University, and Veterans Administration, Nashville, TN 37240.

Using a two choice drug discrimination paradigm, rats (n=72) were trained to discriminate cocaine (15 mg/kg, I.P.) from isotonic saline. Training was done in two stages, starting at 4 mg/kg, then increasing to 15 mg/kg. Rats were tested with two different drug concentrations at each stage. Stimulation of SI cortical neurons resulted in a dose dependent range of effects. Cocaine was found to alter routing of sensory information within a neuronal ensemble. The differential response to cocaine was observed in the somatosensory cortex (SI) and thalamus (VPL) of freely moving rats. Single unit responses were recorded for 10 min. before, and 40 min. after cocaine administration (0.25, 1.0 or 10 mg/kg; i.p.). Procaine (1.0 and 10.0 mg/kg) as well as saline were used in control experiments. Cocaine at 1.0 mg/kg facilitated neuron responses to glutamate evoked and spontaneous firing (71%, n= 7). A similar range of dose dependent effects was observed in cerebellum, however no absolute potentiation by either an absolute potentiation of evoked discharge (25%) or by a relatively weak suppression of evoked discharge (75%) was observed. At higher doses (greater than 300 mg/kg), cocaine caused an overall decrease in neuronal activity. Overall, these data indicate that cocaine can mimic previously observed modulatory actions of NE, and as such may facilitate synaptic excitation transmission within the cerebral cortex and cerebellum. Such actions in the other cortical sensory maps (9), the NSC could provide a physiological basis for cocaine's psychostimulant properties. (Supported by AFSOR-87-0138 & NS18081 to B.D.W.)

PHYSIOLOGY OF ABUSE POTENTIAL SUBSTANCES IN CENTRAL NEURONAL MECHANISMS: SIMULTANEOUS RESPONSES ON SOMATOSENSORY, CEREBELLAR NEURONAL RESPONSES TO IONTOPHORETICALLY APPLIED GLUTAMATE. C.A. Jimenez Rivera and B.D. Kazee. Department of Physical and Biological Sciences, Vanderbilt University, Nashville, TN 37240.

Cocaine's psychostimulant and reinforcing properties, are well known. However the basic physiological mechanisms(s) through which cocaine produces these effects have not been established. Biochemical studies indicate that cocaine can increase central levels of monoamines through blockade of reuptake mechanisms. Previous work from our laboratory has suggested a modulatory role for norepinephrine (NE) in the cerebral cortex and cerebellum of mammalian brain. The goal of the present investigation was to determine to what extent cocaine might exert similar modulatory actions in neuronal circuits with well characterized neurotransmitter systems. Sensory and cerebellar unit activity were recorded from halothane or urethane anesthetized rats. Excitatory responses of individual neurons to iontophoresis pulses of (10^-5) M glutamate (1.5 mEq) were examined, before, during and after cocaine administration. Application of cocaine to somatosensory cortical cells produced a dose dependent range of effects, from marked potentiation of glutamate induced excitation to suppression of both spontaneous and evoked activity. At lower cocaine concentrations (less than 300 mg/kg) the reinforcing effect of cocaine was to enhance the firing rate and more consistent with those mediated by procaine. Overall, these data indicate that cocaine can mimic previously observed modulatory actions of NE, and as such may facilitate synaptic excitation transmission within the cerebral cortex and cerebellum. Such actions in the other cortical sensory maps (9), the NSC could provide a physiological basis for cocaine's psychostimulant properties. (Supported by AFSOR-87-0138 & NS18081 to B.D.W.)


This study defined the differential effects of ketamine, ethanol, and barbiturates on somatosensory (SI) cortical neurons simultaneously recorded in behaving rats. Assays of 25S microsomes (8) were chronically implanted in the SI cortical forepaw region. Discriminated single units from each wire were recorded for time periods (several days or weeks) sufficient to allow multiple drug experiments to be carried out on the same group of neurons. Neurons were categorized according to their sensory and behavioral properties, and also their dose responses to central administration of different drugs. Treadmill running (6 sec ON-45 sec OFF) was used as a standard means of measuring drug effects on locomotor behavior during rest and movement behavior. Subanesthetic doses of ketamine (5-50 mg/kg, I.M.) caused an increase in firing rate in 25, in decreases in 24 of the total 66 neurons which were used in multiple drug experiments. While the animals exhibited motor hyperactivity, confusion and disorientation, this hyperactivity could not explain the firing rate increases as shown by the recorded SI cortical neurons, many of which were excited by similarly analgesic doses of cocaine (1.0, 5.0...25 mg/kg) and three doses of haloperidol (0.25, 0.125, 0.06 mg/kg) during 5 min extinction sessions.

Cocaine (10 mg/kg, I.P.) was administered to single dose of cocaine (30 mg/kg) and independent groups were tested either 4, 5, 6, 12, 16, 18 or 24 hours later. Percent responding on the cocaine lever gradually decreased over time, such that by 8 hours, much of the response was equal on both levers, and by 16 hr, responding on the cocaine lever was 86%. Over longer intervals, there was a gradual return to predrug baseline.

With a single injection of 40 mg/kg cocaine, a similar biphasic effect was observed. At 24 hrs post cocaine, the Ss made 17% of their responses on the cocaine lever (i.e. 83% on haloperidol lever) before returning to predrug baseline. These data provide evidence for a postcocaine withdrawal, during which subjects respond in a manner opposite that produced by the primary drug effect. We believe the biphase-drug discrimination paradigm is an appropriate animal model for the study of physiological substrates which mediate the biphase effect of cocaine in humans.


To define the neural circuit basis for cocaine's effect on cognitive and motor functions, we used multiple electrode recording to examine, before, during and after cocaine administration, neuronal activity in the somatosensory cortex (SI) and thalamus (VPL) of freely moving rats. Up to 12 single neurons were recorded simultaneously through 24um microwires implanted in the forepaw regions of the SI cortex and VPL thalamus. Movement dependent changes in sensory transmission were tested by generating post-stimulus time histograms of neuronal responses to forepaw stimulation through implanted microwires, during treadmill locomotion (20 cm/sec ON-45 cm/sec OFF). Single unit responses were recorded for 10 min. before, and 40 min. after cocaine administration (0.25, 1.0 or 10 mg/kg, I.P.). Cocaine (1.0 and 10.0 mg/kg) as well as saline were tested in control experiments. Cocaine at 1.0 mg/kg facilitated neuron responses to forepaw stimulation in VPL thalamus and SI cortex during both rest and movement, and thus, counteracted movement-induced suppression of sensory responsiveness. By contrast, higher doses of cocaine (10.0 mg/kg) suppressed sensory responses at rest and also enhanced the suppression of sensory responses caused by movement. Saline as well as the lowest dose of cocaine (0.25 mg/kg) did not alter the magnitude of sensory responses during rest or running. Procaine primarily exhibited depressant effects on sensory transmission. The simultaneous recording of many neuron ensembles also allowed study of latency relationships and functional synaptic interactions, through use of spike-triggered histograms. Cocaine was found to alter routing of sensory information within a neuronal ensemble and also abolished the cyclo-oxyring of cortical neurons that was normally observed during rest. In conclusion, our study identifies a major new avenue of investigation for drugs of abuse in awake animals and, moreover, these results are in good agreement with the dose-related modulatory effects of cocaine on synaptic transmission as observed in anesthetized rats. Supported by AA0069, AA0686, and NSBF NSB418979 to JKC, AFSOR-87-0138 and NS18081 to BDW.

POSSIBLE GENETIC PREDISPOSITION TO COCAINE TOXICITY. R.B. Miller, H. Nozakawa and C. Hagen. Dept. of Chemistry, Univ. of Oklahoma, Norman, OK 73019.

The normal human metabolism of cocaine involves ester cleavage by serum and other cholinesterases (Stewart et al., Life Sci., 40, 1557 (1977)). Thus, the existence of genetically atypical individuals should cause concern with respect to their ability to withstand acute or repeated doses of cocaine, as is commonly encountered in the abuse of this substance. This is of particular concern for individuals who are reported to use weapons for the so-called "silent" gene. Such persons exhibit little or no butyrylcholinesterase activity (Dominec et al., Proc. Eur. Congr. Anesthesiol, 2, 186-192, 1968), E.L. Liddell et al., Nature, 193, 561 (1962).

We, thus, decided to examine the tolerance of mice to repeated doses of cocaine following pretreatment with a relatively selective inhibitor of serum cholinesterase, disopropylfluorophosphate (DFP). Folds et al., Clin. Pharmacol. Ther., 7, 620 (1966). In one such test, controls (n=8) were treated 24 hr. prior to cocaine with isotonic saline while experimentals (n=7) received 6.3 mg/kg DFP, i.p. Both groups were subsequently repeatedly injected with cocaine (15 mg/kg, i.p., every 5 min). The mean (±SEM) number of injections required to cause expiration was: controls, 8.5±1.8; experimentals, 3.9±1.1 (P<0.001).
388.15 EVIDENCE OF A GABA/BENZODIAZEPINE MECHANISM MEDIATING PENTYLENETETRAZOL-LIKE STIMULUS PRODUCED DURING COCAINE WITHDRAWAL. D.M. Wood, P.R. Laraby and H.J. Lai. Department of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76110

Chronic cocaine administration results in the development of tolerance to cocaine's reinforcing and psychomotor stimulant effects. Tolerance may be due to changes in the CNS or peripheral sites that mediate the reinforcing properties of cocaine. We examined whether the reinforcing effects of cocaine could be mediated by GABA/Benzodiazepine receptors using pentyleneterazol (PTZ), a GABA antagonist, as a discriminative stimulus (Wood and Lal Life Sci. 41:1431, 1987). In the present experiment, rats were trained to discriminate the stimulus properties of PTZ 20 mg/kg, using a food-rewarded 2-choice lever task. Responding under an FR10 schedule was reinforced on one lever following PTZ injection and on the other lever following saline injection. Following substitution tests were performed, and rats selected the PTZ-appropriate lever after PTZ in a dose-dependent manner, but not after cocaine and saline injections were paired. Cocaine, 20 mg/kg/i.p., was administered for 7 days. Chronic cocaine injections were then terminated and spontaneous withdrawal was assessed by determining if saline would substitute for PTZ. The rationale for this test was that if withdrawal from cocaine produces a PTZ-like stimulus, then subjects injected with cocaine would select the PTZ-lever rather than the saline-lever. Cocaine withdrawal progressively substituted for the PTZ-stimulus reaching a peak (80% PTZ-lever selection) 5 days post-termination of drug. The benzodiazepine, diazepam (1.25-5 mg/kg), but neither the tricyclic antidepressant, imipramine (5-20 mg/kg), the serotonin antagonistic, butaclamol (3.5-10 mg/kg) nor the dopamine receptor agonists, apomorphine (0.25-1 mg/kg) or amphetamine (0.1-0.5 mg/kg) could substitute for PTZ. These data are consistent with the lack of differential response to dopaminergic agonists. We determined the potencies of a series of cocaine derivatives to inhibit [3H]-mazindol binding in order to determine the molecular requirements for their interaction at this site. The affinities of these compounds were substantially diminished by: I) isomerisation to d-enantiomers; II) epimerization of the tropine carbon C-2 substituents; and III) hydroslysis of the C-2 or C-3 substituents to more polar forms. Moderate reductions in affinity resulted from I) quaternization of the nitrogen; II) replacement of the C-3 aromatic substituent with hydrogen, and 3) methylation of the C-3 aromatic ring. Monocyclic and linear chain derivatives of cocaine incorporating the nitrogen and C-3 aromatic substituent also exhibited only moderate decreases in affinity. Modifications which increased affinity or resulted in little change were I) replacement of the C-3 substituent with hexamethylenedioxy or 2-N-demethylation. In summary, binding of cocaine derivatives to the mazindol site on the dopamine transporter appears to require the 1-isomeric form, including the nitrogen and C-3 aromatic substituents.
FEEDING AND DRINKING VI

389.1 THE EFFECTS OF AMPHETAMINE ON FOOD INTAKE AND BODY WEIGHT IN RATS ALLOWED 24-HOUR ACCESS TO FOOD. J.R. Jones* and W.E. Caull. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Recent investigations of tolerance to amphetamine's anorectic effect (Caull, et al., Behav. Neurosci. 105:441-450, 1988) have suggested the importance of assessing both within- and day-to-day body weight changes as well as food intake of rats allowed continuous access to food in order to understand the role of homostatic adaptive mechanisms. In this experiment, independent groups of rats maintained with a LD 12:12 light cycle were injected for 10 consecutive days with either 0, 1, or 2 mg/kg d-amphetamine sulfate either one hour after the lights came on or immediately before the lights were turned off. The results showed that “Daytime” injections produced no effects on daily food consumption, however, the drug-treated rats lost weight over days. “Nighttime” drug injections produced increased daily consumption yet day-to-day body weights were comparable to controls. Further analysis of intake and body weight in terms of two 12-hr periods each day, provides a better understanding of amphetamine's effect on eating, homostatic adaptive mechanisms, and how these factors interact with the circadian organization of eating.


The effect of deprivation level on the development of tolerance to amphetamine anorexia was investigated. The neurochemical consequences of tolerance development were also examined. Seventy-three rats, sensitive to amphetamine (AMP) anorexia, were divided into 6 groups. Three groups were maintained at 85% ad lib. weight while 3 groups had free access to food (100% groups). Three training groups, a contingent (CONT), noncontingent (NONCON) and saline (SAL), each comprised a 100% group and an 85% group. The CONT groups received 3 mg/kg AMP 20 min before 30 min access to milk on Day 1 and saline on Day 2. The NONCON groups received saline 20 min before 30 min access to milk on Day 1 and saline on Day 2. 2. The CONT 85% group consumed significantly more than any other group while the CONT 100% group showed no tolerance to AMP anorexia. At the time of the AMP anorexia test half of the 85% groups were sacrificed 20 min after injection of 3 mg/kg AMP. Later NE, DA, 5-HT, DOPAC, HVA, & 5-HIAA levels were assessed in several brain areas. Preliminary analyses revealed no consistent differences between the CONT and NONCON groups. The failure of the 100% CONT group to develop tolerance demonstrates that food deprivation is essential to the development of tolerance to amphetamine anorexia.

389.3 PERIPHERALLY OR CENTRALLY ADMINISTERED D-AMPHETAMINE INCREASES THE INTAKE OF CHOW SWEETENED WITH SUGAR BUT NOT SACCHARIN. Evans, K.R. and Vaccarino, F.J. Dept. of Pharmacology, University of Toronto, Canada.

We have previously shown that when faced with a choice of different food types, low doses of peripherally or intra-nucleus accumbens (N.Acc.) administered d-amphetamine (AMP) preferentially increases the intake of palatable chow food only as compared to saccharin food. The present study examined whether this AMP-induced/sucrose-selective increase in food intake was related to sweetness or some post-ingestional effect of AMP. Rats were presented with powdered chow and chow sweetened to an equal degree with either sucrose or saccharin following treatments with systemically administered AMP (0.25 mg/Kg) or intra-N.Acc. AMP (0.5 ug or 2.0 ug). Baseline food intake did not differ in the sweetened chow conditions. AMP significantly increased intake of chow sweetened with sugar but had no effect on intake of unsweetened chow or chow sweetened with saccharin. Results suggest post-ingestional factors may be important with respect to AMP-induced feeding. Alternatively, AMP may cause animals to be more sensitive to any aversive properties saccharin might have. Further, the nucleus accumbens supports these effects, consistent with the view that this site is critical in the expression of the facilitatory effect of AMP on feeding.

389.4 SERTRALINE, A 5HT UPTAKE INHIBITOR, INHIBITS FEEDING AND BODY WEIGHT GAIN IN RODENTS. J. Van der Wiele and G. H. Kuiper*. Pfizer Central Research, Deps. Neuroscience and General Pharmacology, Groton, CT 06340.

An extensive literature implicates brain serotonin in the regulation of energy balance (Blundell, Neuropsychopharmacology 23:1537, 1994). More recently, evidence has been gathered implicating serotonin uptake inhibitors to reduce body weight in laboratory animals and obese humans. Sertraline is a potent and highly selective inhibitor of serotonin uptake (Koe et al., J. Pharmacol. Exp. Ther. 220:886, 1983) and we have conducted a preliminary study to assess its effects on feeding and body weight in rodents.

Sertraline decreased food intake by 25-70% and body weight up to 28% in normal mice without any effect on locomotor behavior. Similar effects were observed in normal rats, genetically obese ob/ob mice and ffa rats. These effects developed rapidly after initiation of sertraline treatment and were maintained during 15 days of continued drug administration. Non-specific disruption of behavior does not account for the observed effects on feeding and body weight since the sertraline treated animals appeared healthy and their locomotor behavior was unaffected.

Thus, these results are in accord with a growing body of evidence suggesting that selective serotonin uptake inhibitors may be clinically useful agents for managing some obesity patient populations.


Fluoxetine is a relatively specific serotonin uptake inhibitor which may produce weight loss. In order to investigate this phenomenon, we compared the effect of fluoxetine on weight and eating symptoms in 2 groups of bulimic individuals: a normal weight group (N=11) and a group (N=5) of individuals greater than 10% above ideal body weight. All patients met DSM-III-R criteria for bulimia nervosa, were treated a minimum of 8 weeks, were on no structured meal program, and had a minimum of 1 week separation between fluoxetine injections. The overweight group was more likely to show improvement in symptoms, with all 5 attaining at least a 75% reduction in symptoms while normal weight bulimsics, 4 of 11 (36%) displayed similar improvement (X=8.785; p<0.04). Fluoxetine appeared to have a modest weight loss effect on the overweight group, with the mean weight decreasing from 144.5 pounds (SD=38.6) to 141.8 pounds (SD=38.8) (p<0.07 by paired t-test, two tailed). We expected that there would be a relatively low weight loss and decreased binging and purging, but this was not observed. Weight change was not related to outcome as determined by decreased binging (ANCOVA p>0.83), nor was weight change related to intravenous groups (t-test for independent samples two-tailed, p<0.55). The implications of these findings will be discussed.

Intraventricular injection of neurotensin (NT) suppresses feeding in food-deprived rats (Luttinger et al., Eur. J. Pharmacol., 81, 1982, 499). The neuroanatomical loci that mediate this effect have not been well defined. The present study was conducted to determine whether NT receptors in the SN may be involved in feeding. Male Sprague-Dawley rats were obtained for 18 hours prior to receiving a bilateral intranigral injection of NT (2.5, 5.0, or 10.0 ng/0.5 ul saline) or saline (0.5 ul). Food consumption was measured at 15 minute intervals for 2 hours after injection. Neurotensin suppressed food intake (p<0.05) during the first 15 minutes at the high and low NT doses, and at the intermediate dose. During the second 15 minute interval only the high dose of NT suppressed feeding. No effects on feeding were observed after 30 minutes. Intranigral injection of NT at these doses produced no behavioral stereotypes or other signs of general motor impairment. These results suggest that the SN may mediate the hypophagic effect of NT. (Supported by USPHS grant HD-21560).


Intraneural injection of neurotensin (NT) suppresses feeding in food-deprived rats (Luttinger et al., Eur. J. Pharmacol, 81, 1982, 499). The neuroanatomical loci that mediate this effect have not been well defined. The present study was conducted to determine whether NT receptors in the SN may be involved in feeding. Male Sprague-Dawley rats were obtained for 18 hours prior to receiving a bilateral intranigral injection of NT (2.5, 5.0, or 10.0ng/0.5ul saline) or saline (0.5ul). Food consumption was measured at 15 minute intervals for 2 hours after injection. Neurotensin suppressed food intake (p<0.05) during the first 15 minutes at the high and low NT doses, and at the intermediate dose. During the second 15 minute interval only the high dose of NT suppressed feeding. No effects on feeding were observed after 30 minutes. Intranigral injection of NT at these doses produced no behavioral stereotypes or other signs of general motor impairment. These results suggest that the SN may mediate the hypophagic effect of NT. (Supported by USPHS grant HD-21560).


Rats ingest liquid diets by licking a drinking tube in bursts separated by pauses. This study determined the effect of selective dopamine D1- and D2 receptor antagonists on the size of bursts (SB) and the length of the interburst interval (IBI). Male albino Sprague Dawley rats were trained to drink a highly palatable test diet (0.006M saccharin solution, 25 ml/kg) for 30 minutes. Time of tongue contact with the drinking tube was measured to the nearest 10 msec by an electronic drinkometer. SDR2390, a selective dopamine D1 receptor antagonist at doses of .012 to .015 mg/kg (s.q.) reduced volumetric intake in a dose related manner by increasing the IBI and the number of bursts sufficient to compensate for a significant increase in SB. Liquipinole, a selective dopamine D2 receptor antagonist at doses from .03 to .16 mg/kg reduced volumetric intake in a dose related manner principally by decreasing the size of the burst. IBI and number of bursts were significantly altered (increased and decreased respectively) only at the highest dose. The inactive enantiomers of both compounds were ineffective in altering volume intake or the two parameters. We conclude that antagonism of the dopamine D1 receptor has a different effect on ingestive behavior than does D2 receptor antagonist.


This study assessed the feeding response of insulin treated male SDR and matched controls (n=6 each) to: 1) SCH 23390 (SCH) a D1-receptor antagonist, and 2) novelty stress (NOV). All animals were entrained on a restricted 12-hrs of food deprivation, followed by treatment, presentation of test meal, and behavioral monitoring (for 30 min). In the SDR, SCH (100 ug/kg) incurred a 62% and 27% increase in meal size respectively, as compared to a 1% and 29% increase in controls. Conversely, SCH (100 ug/kg) reduced the meal size by 72% and 30% in the SDR and controls respectively. NOV increased the meal size of SDR and controls by 43% vs 24% respectively. Interestingly, at low doses SCH elicited grooming but blocks it at high doses. A similar inverted u dose-response was seen here, with low doses of SCH stimulating and high doses inhibiting the feeding response. We reported earlier that the SDR displays an increased sensitivity to SKF 38393 and novelty stress-induced grooming. In this case, the dose-response curve of the SDR appears to have shifted to the left, again implicating increased sensitivity of the SDR to D1 receptor and stress associated responses.


Central dopaminergic (DA) systems are thought to participate in the regulation of feeding. The activating effects of SKF 38393 on rats sham feeding sucrose (SUC) and corn oil (CO) in rats. We examine the effect of the selective D1- and D2 receptor antagonists, SCH23390 (SCH) and SCH389.8, on spontaneous intake, of 100% CO and 10% SUC in CO rats. Results V values are mean ± SEM of 5<10 inhibition of sham intake + SDME.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>V (mM)</th>
<th>CO (mM)</th>
<th>SUC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>.050</td>
<td>.012</td>
<td>.025</td>
</tr>
<tr>
<td>D2</td>
<td>.100</td>
<td>.047</td>
<td>.055</td>
</tr>
<tr>
<td>D1</td>
<td>.200</td>
<td>.100</td>
<td>.100</td>
</tr>
<tr>
<td>D2</td>
<td>.300</td>
<td>.150</td>
<td>.150</td>
</tr>
<tr>
<td>D1</td>
<td>.400</td>
<td>.200</td>
<td>.200</td>
</tr>
<tr>
<td>D2</td>
<td>.500</td>
<td>.250</td>
<td>.250</td>
</tr>
</tbody>
</table>

Note: Tests were conducted under the following 12-h food deprivation, N=12, doses are mg/kg,i.p., given 15 min before SUC or CO. *drug vs veh, **CO vs SUC, p<.01. CONCLUSION When SF CO rats are pretreated with D1 and D2 antagonists than when SF SUC. It is not clear whether this difference in sensitivity is due to a greater release of DA during CO SF than during SUC SF, or to the possibility that SF CO may be less dependent on DA systems than the SF of SUC. Neurochemical studies are needed to distinguish between these possibilities. (Supported by NHHS459 (GFS).)

Rats shan feed (S) corn oil (CO) and sucrone (SU) in a concentration-dependent manner, suggesting that the osmotic stimuli of these solutions are positively reinforcing. Since the system of this study is to determine the rank order of preference of 100% CO, 50% CO and 100% CO in the absence (SFing; cannula open) and presence (real feeding; cannula closed) of post-feeding cues.

Three groups of overnight food-deprived rats were trained to SF 2 solutions in alternate 1-bottle tests. Groups 1, 2, 3 were trained to SF 100% CO; 100% CO and 10% SU; and 10% SU and 6% SUC, respectively. Rats then received three 10-min, 2-bottle preference tests. Following the SFing preference tests, rats were given three 30-min, 2-bottle preference tests with the cannula closed. During SFing the rank order of preference was 100% CO >> 50% SUC >> 6% SUC. In contrast, when the cannula was closed, order of preference was reversed.

The results show that α-OA injected into the PVN elicits feeding. This effect occurs via an action at α2 receptors, and appears to be mediated by the α2 receptor subtype underlying natural feeding processes.

FEEDING AND DRINKING VI

389.14


Administration of norepinephrine (NE) into the paraventricular nucleus (PVN) produces eating via the activation of α2-noradrenergic receptors. Studies suggest that NE level stimulates hypothalamic nuclei (including PVN) of Zucker obese vs. lean rats, and therefore may contribute to the development of obesity. The present study examines the density of α- and α2-noradrenergic receptors in discrete hypothalamic and extra-hypothalamic areas of lean and genetically obese rats.

Patterns of food intake and macronutrient selection are known to vary as a function of time of day and addiction. The present study examines the density of α- and α2-noradrenergic receptors in discrete hypothalamic and extra-hypothalamic areas of lean and genetically obese rats.

The trace amine octopamine (OA), which occurs naturally in mammalian brain, has been reported to possess significant actions on nondopaminergic systems. The present experiments were designed to examine the effects of OA on the intake of the rats at different times of day on feeding behaviour in satiated rats following injection into the PVN, and to compare these effects elicited by norepinephrine (NE).

α-OA induced a dose-dependent increase in food intake which was maximal at 1 μg/μl, and did not increase further. Intake was greater when the rats drank the non-nutritive solution than when they drank the nutritive solution.

The results show that α-OA injected into the PVN elicits feeding. This effect occurs via an action at α2 receptors, and appears to be mediated by the α2 receptor subtype underlying natural feeding processes.
390.1


Rats with neonatal destruction of dopamine (DA)-containing neurons in the nigrostriatal pathway were challenged with acoustic stimuli to determine if the dopaminergic neurons produce acute decreases in motor activity and responsiveness to stimuli. To study the sensorimotor effects of haloperidol (HP), rats were tested for conditioned responses to auditory stimuli presented at varied intervals. In different experiments the intensity or duration of the stimulus was varied, and food-deprived rats were rewarded for responding during the stimulus. HP suppressed responding to both high and low intensity stimuli equally. In contrast, the anticholinergic atropine had greater effects at the low intensity stimulus. HP also had a greater effect if the rats were required to respond within 2 rather than 5 sec. In a second series of experiments, food-deprived rats were tested in 30-min feeding sessions. As well as measuring food and water intake, an observer kept real-time event records of feeding, drinking, and rearing. HP or 6-OHDA lesions decreased food and water intake, total feeding, rate of feeding, average duration of periods of feeding, and rearing. Rearing was significantly increased after the 6-OHDA lesion, and this effect was partially reversed by HP.

390.2

CHARACTERIZATION OF THE MOTOR AND SENSORMOTOR FUNCTIONS OF BRAIN DOPAMINE (DA) ANTAGONISTS OR LESIONS OF BRAIN DA NEURONS PRODUCE ACUTE DECREASES IN MOTOR ACTIVITY AND RESPONSIVENESS TO STIMULI. TO STUDY THE SENSORIMOTOR EFFECTS OF HALOPERIDOL (HP), RATS WERE TESTED FOR CONDITIONED RESPONSES TO AUDITORY STIMULI PRESENTED AT VARYING INTERVALS. IN DIFFERENT EXPERIMENTS THE INTENSITY OR DURATION OF THE STIMULUS WAS VARYED, AND FOOD-DEPRIVED RATS WERE REWARDED FOR RESPONDING DURING THE STIMULUS. HP SUPPRESSED RESPONDING TO BOTH HIGH AND LOW INTENSITY STIMULI EQUALLY. IN CONTRAST, THE ANTICHOLINERGIC ATROPINE HAD GREATER EFFECTS AT THE LOW INTENSITY STIMULUS. HP ALSO HAD A GREATER EFFECT IF THE RATS WERE REQUIRED TO RESPOND WITHIN 2 RATHER THAN 5 SEC. IN A SECOND SERIES OF EXPERIMENTS, FOOD-DEPRIVED RATS WERE TESTED IN 30-MIN FEEDING SESSIONS. AS WELL AS MEASURING FOOD AND WATER INTAKE, AN OBSERVER KEPT REAL-TIME EVENT RECORDS OF FEEDING, DRINKING, AND REARING. HP OR 6-OHDA LESIONS DECREASED FOOD AND WATER INTAKE, TOTAL FEEDING, RATE OF FEEDING, AVERAGE DURATION OF PERIODS OF FEEDING, AND REARING. REARING WAS SIGNIFICANTLY INCREASED AFTER THE 6-OHDA LESION, AND THIS EFFECT WAS PARTIALLY REVERSED BY HP.
390.7 Dopamine Concentration Increases in the Cerebral Cortex and Brain Stem of the Hibernating Little Brown Bat (Myotis Lucifugus), W.A. Baumont, H. Scharf, C.A. Delavert, and A. Hashtip (SPHR: B. Scharrer). Veterans Administration Medical Center, Bronx, NY and Center for Neurochemistry, Ward's Island, NY.

Hibernation (H) is an adaptive orchestrated physiologic response to harsh environmental conditions. Inhibitory changes in various bioamines during this behavior may afford insight into the mechanism of H and temperature regulation in general. To determine the effect of H on the concentrations of dopamine (DA) and its metabolite DOPAC in the cerebral cortex (CC) and brain stem (BS), we sacrificed euthermic nonhibernating (NH) bats in October and H bats (core body temp 6-20°C) in April. The brain was carefully dissected into CC and BS and stored at -50°C until extraction and assay of the neurotransmitters using HPLC with electrochemical detection. Results are expressed as mean ± SEM:

<table>
<thead>
<tr>
<th>State</th>
<th>CC</th>
<th>BS</th>
</tr>
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<tbody>
<tr>
<td>NH</td>
<td>15.2 ± 3.50</td>
<td>4.0* ± 0.78*</td>
</tr>
<tr>
<td>H</td>
<td>35.3 ± 3.90*</td>
<td>5.9 ± 0.78*</td>
</tr>
</tbody>
</table>

All concentrations are expressed in ng/mg protein

*p < 0.01, +p < 0.01

Conclusions: (1) CC DA and DOPAC concentrations markedly increased during H, (2) BS DA increased during H, and (3) the increase in DA concentration in the brain may be a generalized finding during H.

390.9 Ascorbate Infusions into the Neostriatum Modulate Components of the Behavioral Response to Acute and Chronic Amphetamine, G.V. Rebec, A. Basse-Tomusk, & M. Lam. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Systemic, intraventricular, or intrastriatal ascorbate (AA) attenuates the behavioral response to amphetamine and potentiates the effects of haloperidol (e.g., White et al., Psychopharmac., 94: 284, 1988). Because the neostriatum plays a key role in the behavioral response to these drugs, AA may exert its antianphetamine effect in this site. To test this hypothesis, we infused AA (2.0 µg/ul) or saline (0.9%) bilaterally into the neostriatum of rats at a rate of 0.4 µl/min and monitored the behavioral response to 1.0 mg/kg d-amphetamine administered alone or preceded by 0.025 mg/kg haloperidol. Infusions of AA into the neostriatum attenuate components of the behavioral response to amphetamine and enhance the anticoital effects of haloperidol. Thus, AA appears to antagonize dopamine transmission in the neostriatum. Supported by DA 02451 and BNS 87-11240.

390.11 Caffeine-Induced Conditioned Place Preference and Aversion, H.J. Poschel, E. Blanke, and J.A. Rensinger. Dept. Psychology, Queen’s University, Kingston, Canada.

Although caffeine may be the most widely used behaviorally active drug, few studies have illustrated its reinforcing properties. The place conditioning paradigm has been used to illustrate the reinforcing properties of many drugs of abuse, such as the dopamine (DA) agonists cocaine and amphetamine. Following several pairings of a drug injection with one side of a dual-chambered box, the undrugged chamber displays a preference for the drug-paired side. The present study utilized conditioned place conditioning to assess the reinforcing properties of caffeine in male Wistar rats. Results indicate that a high dose (30 mg/kg IP or SC) produced a significant place aversion, whereas lower doses (10.0 mg/kg IP) produced place preference. Using an identical procedure, (+)-amphetamine (2.0 mg/kg IP) produced a significant place preference. These results suggest that doses of caffeine produce a biphasic effect on place conditioning, and are consistent with in vivo electrochemical evidence (Morgan, Dunn, & Vetalis, Neurochem. Abstr. 13:253, 1978) which suggests that low caffeine doses increase and high doses decrease caudate DA release. (Supported by NERC).

390.12 Evidence for OpioiD-Dopamine LINK IN A NEW ANIMAL MODEL OF MANIA, W. Fratta, P. Fadda*, M. Martellotta*, G. L. Gessa*. Department of Neurosciences Bernard B. Brodie, University of Cagliari, Italy.

Sleep deprivation (S.D.) often precedes the onset of mania. Sleep deprived rats show a stage of excitement before they fall asleep that has striking similarities with symptoms of mania, such as insomnia, hyperactivity, aggressiveness and hypersexuality. To verify if such a response might represent a valid model of mania, we studied the effect of different drugs on the excitement following 72 hrs S.D. in rats. Wherever the duration of the excitement and EEG arousal was markedly shortened by chronic lithium, by haloperidol (0.2 mg/kg) and SCH 23390 (at the dose as low as 3 &mu;g/kg) but was not influenced by (-) sulpiride (25 mg/kg).

Moreover, S.D. induced excitement was depressed by Haloxone and markedly prolonged by morphine and other opioids. The results suggest that S.D. induced "mania-like" behavior is mediated by the activation of an opioid-dopaminergic link leading to D1 receptor stimulation.

390.13 Enriched and Impoverished Environments: Effects on the Turnover Rates of Monoamine Neurotransmitters, N. J. Renner (Department of Psychology, University of Wisconsin, Oshkosh, WI 54901), C. L. Blank, & K. Freeman (Department of Chemistry, University of Oklahoma, OK 73109).

Previously, we reported data concerning tissue concentrations of monoamine transmitters and their metabolites in rats after enriched and impoverished housing. The present study extended those studies by examining turnover rates of these transmitters. Sprague-Dawley male rats (27-wk-match pair, 70 days) were randomly assigned to either an enriched condition (EC); group housing, 75x5x50 cm cage, several objects, rotated daily on an inclined environment (IE); solitary housing, small cage, no cagemates), for 30 days. So were injected with 200 mg/kg of the L-aromatic amino acid decarboxylase inhibitor NSD-1015, 30 min, and sacrificed by 800 msec of 10 KHz irradiation to the head at 2,45 GHz (New Japan Radio NJR-2603). Brains were dissected into 11 sections and analyzed via HPLC-EC using a reversed phase column packed with 3 µm particles (Lin., et al., J. Lig. Chromatog., 1984, 7(3), 509-538). IC significantly increased EC in dopaminal transmitter turnover rate (5-HTP buildup; p < 0.001). Dopamine turnover (Dopa buildup) was significantly different in only one of the two replications (p < 0.04). In occipital cortex, the region of largest EC-IC anatomical differences, no significant differences were found. These findings are opposite the direction of other brain differences reported for EC-IC comparisons.
MONOAMINES AND BEHAVIOR

By B. Swedan*, H. Edinger and A. Siegel, Depts. of Neurosciences and Physiology, UMDNJ, Newark, NJ, 07103.

The medial preoptic-anterior hypothalamus (mPO-AH) from the rat, since dopaminergic (DA) fibers supply this region, we investigated the contribution of DA mechanisms in the mPO-AH to DA facilitation of AD. These effects of mPO-AH and APOM insertion were determined by examining the changes in central dopamine (DA) activity and levels which varied across strains of mice. It has been shown that combined tail-shock and immobilization depletes norepinephrine in the rat hypothalamus, and depresses locomotor and exploratory behavior. Oral tyrosine ingestion blocks this behavioral depression. Since oral Aspartame also produces increased blood levels of tyrosine it ought to have an effect similar to tyrosine alone. This study had two purposes: A) To determine whether immobilization stress done without prior tyrosine ingestion post-stress behavior and depression; and B) To determine whether Aspartame ingestion would block post-stress behavioral depression of exploratory behavior. Forty Sprague-Dawley rats were fed high carbohydrate meal dosed with Aspartame (200 mg/kg). Two hours of immobilization stress followed. Immobilization stress depressed all behavior--validating the more humane (non-electric shock) stress stimulus. In the Aspartame condition the post-stress depression was significantly blocked, thus demonstrating a post-stress behavioral effect of Aspartame ingestion not previously reported.


The medial preoptico-anterior hypothalamus (mPO-AH) has been shown to be the origin of the descending fibers mediating hypothalamically elicited affective defense behavior (AD) in the cat. Since dopaminergic (DA) fibers supply this region, we investigated the contribution of DA mechanisms in the mPO-AH to DA facilitation of AD. These effects of mPO-AH and APOM insertion were determined by examining the changes in central dopamine (DA) activity and levels which varied across strains of mice. It has been shown that combined tail-shock and immobilization depletes norepinephrine in the rat hypothalamus, and depresses locomotor and exploratory behavior. Oral tyrosine ingestion blocks this behavioral depression. Since oral Aspartame also produces increased blood levels of tyrosine it ought to have an effect similar to tyrosine alone. This study had two purposes: A) To determine whether immobilization stress done without prior tyrosine ingestion post-stress behavior and depression; and B) To determine whether Aspartame ingestion would block post-stress behavioral depression of exploratory behavior. Forty Sprague-Dawley rats were fed high carbohydrate meal dosed with Aspartame (200 mg/kg). Two hours of immobilization stress followed. Immobilization stress depressed all behavior--validating the more humane (non-electric shock) stress stimulus. In the Aspartame condition the post-stress depression was significantly blocked, thus demonstrating a post-stress behavioral effect of Aspartame ingestion not previously reported.


When food deprived rats were given daily exposures to photocell activity cages, those reared in isolation were hyperactive on the first two days of testing, when compared to socially-reared controls. By day 3 there was no difference between the two groups. However, when testing was paired with food presentation, the locomotor hyperactivity in the isolated rats was reinstated. Determination of brain tissue concentration of monoamines (DA, NE, and 5-HT) and their metabolites from similarly reared, isolated (individual cages) and group-reared rats (6 per cage), indicated a significant reduction in fronto-cortical dopamine activity in isolates. This is in confirmation of earlier studies on the effects of social-isolation on central dopamine function and behavior.


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It has been shown that combined tail shock and immobilization depletes norepinephrine in the rat hypothalamus, and depresses locomotor and exploratory behavior. Oral tyrosine ingestion blocks this behavioral depression. Since oral Aspartame also produces increased blood levels of tyrosine it ought to have an effect similar to tyrosine alone. This study had two purposes: A) To determine whether immobilization stress done without prior tyrosine ingestion post-stress behavior and depression; and B) To determine whether Aspartame ingestion would block post-stress behavioral depression of exploratory behavior. Forty Sprague-Dawley rats were fed a high carbohydrate meal dosed with Aspartame (200 mg/kg). Two hours of immobilization stress followed. Immobilization stress depressed all behavior--validating the more humane (non-electric shock) stress stimulus. In the Aspartame condition the post-stress depression was significantly blocked, thus demonstrating a post-stress behavioral effect of Aspartame ingestion not previously reported.

STRAIN-SPECIFIC CATECHOLAMINE VARIATIONS INDUCED BY STRESSORS: RELATION TO BEHAVIORAL CHANGE. N. Shanks, S. Zalcman*, C.R. Prince*, and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ont. Canada K1S 5B6.

Exposure to inescapable shock provoked region-specific alterations of norepinephrine (NE) and dopamine (DA) activity and levels which varied across strains of mice. In the mesolimbic frontal cortex and nucleus accumbens shock induced an increase of DOPAC accumulation and pronounced reductions of DA in some strains, while in others these variations were less pronounced or entirely absent. Likewise, the stressor-provoked reductions of NE and increases of MHPG accumulation in hypothalamus, hippocampus and locus coeruleus varied appreciably across strains. Strain-specific disturbances of shuttle escape performance associated with inescapable shock correlated relatively well with the MHPG accumulation in the locus coeruleus and the DA reductions in the frontal cortex. Likewise, stressor-provoked alterations of self-stimulation from the nucleus accumbens were related to DOPAC accumulation in this region.


A selective breeding strategy has resulted in rapid, persistent line differences in mice from a common genetic background. NC900 mice exhibit high levels of species-specific, isolation-induction aggression when evaluated in a social interaction test. Covariance aggression, but high levels of immobility. In both lines, even one exposure to the social interaction test decreases the latency to attack. We hypothesized that central DA system influenced by selective breeding, mediated, at least in part, this line difference. Mice from both lines, half of which had been exposed to the social interaction test, were sacrificed four days after testing. Concentrations of dopamine and its acidic metabolites, DopAC and HVA were quantified using microdissected samples from various brain regions. Significant differences for both line and social experience were found but with no interaction. NC100 mice had significantly increased concentrations of DopAC and HVA, in both the caudate nucleus and amygdala. The present results support the occurrence of DA pathway line differences, and also lead to the hypothesis that social experience results in persistent alterations in dopamine utilization, perhaps as described for corticosterone sensitization. (Supported by FHS Grants HD41648 and HD03110.)


Functional heterogeneity of the dopamine (DA) system has typically been studies using localized 6-hydroxydopamine (6-OHDA) lesions and intracranial injections of DA agonists and antagonists. The transplantation of fetal DA cells provides another tool for studying functional heterogeneity of the DA system. In this study, rats were unilaterally lesioned with 6-OHDA and then tested for amphetamine (AMPH)-induced turning with 5 mg/kg methamphetamine. Rate which did not meet a criterion of 35 turns per min were not used. The rats received transplants in the nucleus accumbens (NAC), dorsorostral striatum (DS), and ventral striatum (VS). The effects of DA transplants on AMPH induced turning, locomotion, and stereotypy were observed. Preliminary data showed that transplants caused an increase in AMPH induced locomotion. Transplants in the DS reversed direction of AMPH induced turning. Transplants in the VS increased duration of AMPH induced locomotion and turning and increased the amount of time spent in stationary stereotypy.
A whole night's sleep deprivation improves mood in depressed patients. Nothing is known about the mechanisms involved. Since thyroid disorders have also often profound effects on mood, we investigated changes in TSH during and after sleep deprivation in depressed patients and healthy controls.

In 50 patients with major depression, TSH concentration was 1.4±1.35mU/l at 8 a.m. before and 2.14±1.43mU/l at 8 a.m. the next morning after sleep deprivation. The increase in TSH levels was significantly correlated to depression measured as changes in scores of visual analog mood scales.

TSH secretion is believed to be under central noradrenergic control in the rat. We have investigated the effects of oral prazosine, yohimbine, and propranolol (α, α₂, and β-adrenoceptor antagonists) on TSH secretion, probably induced by sympathetic activation on presynaptic α₂ receptors, thereby leading to enhanced norepinephrine activity. We conclude that at least the observed postprandial effect of SD is mediated by increased noradrenergic activity, probably due to activation of noradrenergic neurons in the locus coeruleus.
CARDIOVASCULAR REGULATION VI

391.5 EFFECTS OF SINOAORTIC NERVE DE NerVATION ON THE RELEASE OF ENDOGENOUS NEUROPEPTIDES FROM THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS OF UNANESTHETIZED FREELY MOVING RATS. J.M. Quay* and C.C. Westfall. (BPSM: S. Korenstein.) Department of Pharmacology, Louis University School of Medicine, St. Louis, Mo. 63104.

We previously reported enhanced basal and K* stimulated release of neuropeptides (NPH) from the paraventricular (PVH) and old SHR as compared to age matched WKY and SD rats. This increased release was attenuated in 12 wk old SHR suggesting enhanced norepinephrine neuronal activity during the developing phase of hypertension. In addition, a reciprocal relationship exists with respect to blood pressure and NE release such that an increase/decrease in blood pressure resulted in a decrease/increase of PVH NE release. These results suggested that the noradrenergic pathways of the PVH contribute to the maintenance of arterial blood pressure homeostasis presumably via the baroreceptor reflex. The present study examined the effects of sinoaortic denervation (SAD) on PVH NE release. The reciprocal relationship between blood pressure and NE release was abolished after SAD. The administration of phenylephrine no longer attenuated the release of NE, and the administration of sodium nitroprusside no longer exacerbated PVH NE release. These results demonstrate that an intact baroreflex is necessary for the noradrenergic pathways of the PVH to contribute to the maintenance of arterial blood pressure homeostasis. (Supported by NIH grants HL35202 and HL6313.)

391.7 LATERAL HYPOTHALAMIC AREA ROLE IN CARDIOVASCULAR CONTROL. E.E. Spofford, W.R. Scartney and A.D. Lemos. Dept. of Neurology and Anatomy and Neurosurgery, The University of Mississippi Medical Center, Jackson, MS 39216.

We have previously reported that L-glutamate stimulation of the lateral hypothalamic area (LHA) in rats lowers both blood pressure and heart rate (Soc. Neurosci. Abst., 11(1): 285, 1987). Stimulation of the subbicular neurons and dorsal zona incerta did not alter blood pressure or heart rate, while ventral zona incerta stimulation generated hypertension and bradycardia. We reported that this LHA influence on blood pressure is mediated by reductions in cardiac output and is not a result of a decrease in total peripheral resistance. J. Neurol. 1, 365-372, 1986.

In our initial studies, we observed a rostrocaudal organization within the LHA. This report extends these studies to discern potential differences after stimulating different subdivisions of the perifornical hypothalamic area. Stimulation of the lateral perifornical LHA (LHA(L)) which lies lateral to the ventromedial nucleus of the hypothalamus, decreased the heart rate and blood pressure. In contrast, stimulating the posterior part of the LHA (LHA(P)), which lies caudal to the LHA(L) and lateral to the mamillary nuclei, decreased the heart rate and blood pressure minimally. Stimulation of the perifornical hypothalamic area caused a fall in blood pressure but no change in heart rate.

The use of pharmacological blockers permitted a determination of whether the LHA(L) bradycardia was sympathetically or vagally mediated. Timolol eliminated the bradycardic response. Atenolol reduced the bradycardic response by 35%.

Our results show that the LHA(L) influences both heart rate and blood pressure either directly or through a central neurohormonal mechanism. The adjacent perifornical region affects blood pressure but not heart rate.


In status epilepticus, mean arterial blood pressure (MAPB) increases during early seizures but decreases during later seizures (Glaser Adv. in Neurotol. 34: 395-398, 1983). Renal blood flow (RBF) was measured during serially induced seizures to determine whether the fall in i.c.b MAPB results from loss of tone in the renal vascular bed. During each of the 20 injections of kainic acid (KA) which evoked seizures, MAPB increased nearly 50% while RBF transiently decreased nearly 100%. With subsequent seizures, the rises in MAPB then remained constant while the decreases in RBF became smaller. RBF became pressure passive when later seizures were accompanied by a decrease in MAPB, i.e., administration of norepinephrine during interictal periods always produced profound renal vasocstriction, indicating that the renal vascular bed remained responsive. The importance of these changes in the renal vascular response to serial seizures will be discussed. (Supported by NIH NS-17443.)


Corticotropic releasing factor (CRF) acts within the central nervous system (CNS) to elevate cardiac output (CO) and decreases heart rate (HR) simultaneously and markedly inhibit reflex bradycardia in response to increased AP. If these cardiovascular changes are mediated by inhibition of sympathetic outflow, the baroreceptors in the CNS were terminally within the CNS, prior removal of such afferents should preclude the ability of CRF to activate additional cardiovascular reflexes. To examine this possibility, the CNS cardiovascular actions of CRF were measured in rats after sinoaortic deafferentation (SAD). All experiments utilized conscious, unrestrained male rats previously instrumented with lateral intracerebroventricular (icv) cannulae and femoral arterial catheters for direct measurement of AP and HR. Sham (n=6) and SAD (n=11) operations were performed in a one-stage procedure twice prior to first exposure of the same rats to CRF. A microinjection of NPY into the hypothalamus and/or femoral arteries elicited a dose-dependent increase in AP and HR. In contrast, icv CRF infusions of 100 nl of 1-glutamate (Glu) into lateral hypothalamus (LH) were studied in conscious male Sprague-Dawley rats (n=7, 360-360g). Mean arterial pressure (MAP), heart rate, blood flow (BF) and vascular resistance (VR) in hindquarter (HQ), renal (R) and mesenteric (M) vessels were monitored. The site of injection and spread were confirmed (Cruz stain and sections (50u) and by a radioactive tracer. Glu (100 nmoH in LH produced a sustained pressor response (+31±3% MAP, p<0.01). The injection site in R became 1-2 mm and subsided in 10-15 min. In 4/7 animals the pressor response was accompanied by an initial decrease in heart rate (-52±18 bpm, p<0.05) and in all animals by a sustained tachycardia (+51±15 bpm, p<0.01). The BP decreased in R and M blood vessels by -28±7% (p<0.01) and -30±12% (p<0.05), due to an increase in VR (+73±34%, p<0.05) and VR (+60±21%, p<0.05). The results demonstrate that in the conscious rat Glu in LH produces pressor and vasoconstrictor responses, not depressor responses as reported in the anesthetized rat. It is suggested that Glu receptors in LH mediate the pressor response evoked by electrical stimulation of the LH area.

391.12 EFFECT OF SYMPATHETIC NERVOUS SYSTEM ON DAILY VARIATION OF CARDIOVASCULAR PARAMETERS IN VARIOUS MONKEYS. M.I. Talan and B.T. Engle. Lab. of Behavioral Sciences, Gerontology Research Center, NIA, Baltimore, MD 21224.

Heart rate, stroke volume, and interarterial blood pressure were monitored continuously in each of four monkeys, 18 consecutive hours/day for several weeks. Mean heart rate, stroke volume, cardiac output, systolic and diastolic blood pressures and total peripheral resistance were calculated for each minute, and reduced to hourly means. After baseline data were collected for approximately 20 days, observation was continued for equal periods of conditions without baroreceptor blockade, with blockade of sympathetic, and double sympathetic blockade achieved by intra-arterial infusion of atenolol, prazosin, or a combination of both in concentration sufficient for at least 75% reduction in response of respective agonists. A stable diurnal pattern of hemodynamic function was observed in the control condition: cardiac output fell throughout the night primarily as a result of a fall in heart rate since stroke volume was relatively unchanged; a small reduction in blood pressure also occurred; however, this fall was buffered by a rise in peripheral resistance which paralleled the fall in cardiac output. Average overnight total peripheral resistance was 45% greater during increased sympathetic nerve activity than during double blockade. The hemodynamic pattern was not eliminated by selective blockade of a- or b-sympathetic receptors, or by double sympathetic blockade; in fact, it was exacerbated by sympathetic blockade indicating that the sympathetic nervous system attenuates these events. The fact that sympathetic blockade did not eliminate this pattern suggests that a diurnal loss in plasma volume may mediate the fall in cardiac output, and that the rise in total peripheral resistance reflects autoregulation of arterial pressure.

The time-course of recovery of arterial baroreflex control of heart rate after unilateral mid-cervical vagotomy was evaluated by measuring the baroreflex sensitivity. Sensitivities were measured at 0.1, 0.3, 0.5, 1.0, and 2.0 Hz using a constant interbeat interval (IBI) during controlled ventilation. These data show that the baroreflex sensitivity returns to normal levels within 1 week after vagotomy and suggests that this is a normal physiological response. These data are consistent with previous findings that the baroreflex is capable of recovering within this time frame. However, it should be noted that this study was performed in rabbits and further investigation is warranted.


These experiments investigated the recovery of neural control of blood pressure (BP) and heart rate (HR) in rats one week after administration of HDHA. The animals were placed in the femoral artery and vein at least 24 hr before experiments. BP was reduced in the HDHA-treated rats (104±4 mm Hg) vs 88±3 mm Hg in controls (p<0.01) whereas heart rate was not significantly different. In plasma samples obtained while the rats rested quietly, norepinephrine (NE) levels were significantly less in the HDHA-treated rats (152±13 pg/ml vs 124±6 pg/ml, p<0.05) whereas epinephrine levels were similar in both groups. The recovery of baroreceptor reflex response may be related to the fact that the baroreflex is capable of recovering within this time frame. These data are consistent with previous findings that the baroreflex is capable of recovering within this time frame. However, it should be noted that this study was performed in rats and further investigation is warranted. These observations warrant further investigation.

391.13 CANINE MYOCARDIAL CELL SURFACE 5-ADENOSINE RECEPTORS: EFFECT OF ALTERED DETERGENT CHOLESTeryl MAXIMAL STELLATE GANGLIONIC STIMULATION. W.M.Watson-Wright* at Wilterson, H.J. Johnson* and J.A.Armouri Dept. of Physiology/Biophysics, Dalhousie Univ., Halifax, N.S.

Electrical stimulation of thoracic sympathetic ganglia results in increased inotropism whereas cardiac cells and then pronounced within a few minutes, even though stimulation continues. This inhibition could be due to a reaction. Guanyl nucleotides negatively modulated the binding of [3H]CGP-12177, to cell surface receptors in tissues which had been removed from normal and ischemic hearts. The results of the experiments in vivo demonstrated that sympathetically mediated responses are mediated by a variety of mechanisms. These observations have important implications for the understanding of the role of the sympathetic nervous system in cardiovascular control.

391.14 DEVELOPMENT OF 5-ADENOSINE RECEPTOR AND RESPONSIVENESS OF ADENYLATE CYCLASE TO ADENOSINE ANTAGONISTS IN EMBRYONIC CHICK HEART. T.A.Blaire and T.F.Murray. Oregon State Univ., Corvallis, OR.

The developing chick heart has been used to study the biochemical events involved in the activation and regulation of various neuroreceptors. In the present study we have used the embryonic chick heart to investigate the development of 5-adenosine receptors and their interaction with adenylate cyclase. These experiments were performed in rats and the results of these previous physiological studies were combined to demonstrate a relationship between the development of sensitivity to adenosine analogues and the development of adenylate cyclase. At 5-7 days in ovo the number of 5-adenosine receptors (5AR) increased from 0.02±0.01 fmol/mg protein to 0.1±0.01 fmol/mg protein. The number of 5AR was increased in the heart from 0.02±0.01 fmol/mg protein to 0.3±0.01 fmol/mg protein at 0.94 fmol/mg wet wt and was not altered after 20 minutes stimulation of 0.33 fmol/mm Hg. This response was still significantly reduced from control at 1 week following vagotomy (1.2±0.24 fmol/mg wet wt). At 6 weeks following vagotomy the baroreflex sensitivity had returned to control levels (3.4±1.22 sec/mm Hg). No changes were found in the response to methyl choline indicating that alterations in the muscarinic cholinergic receptors could not account for recovery of baroreceptor reflex response after vagotomy. These data support the concept that recovery of baroreceptor reflex response may be related to compensatory changes in the intact contralateral vagus nerve and not an alteration of cholinergic muscarinic receptors. Supported by the National Institute of Health HL38317.

391.15 AGONIST RADIOLIGAND INTERACTIONS WITH THE SOLUBILIZED PORCINE ATRIAL ADENOSINE RECEPTOR. H. Leid and I.F. Murray. Coll. of Pharmacy, Oregon State Univ., Corvallis, OR 97331.

The agonist radioligand [125I]HPIA was used to characterize solubilized cardiac adenosine receptors (cADoR). Porcine atrial membranes were solubilized using a mixed detergent system consisting of 1% IGSH and digitonin and 0.08% w/v cholate. [125I]HPIA showed saturable binding to an apparently homogeneous population of solubilized recognition sites with a Bmax of 25±1 fmol/mg protein and a K<sub>G</sub> of (6.9±0.8) x 10<sup>-10</sup> M. The results of kinetic experiments suggest that [125I]HPIA interacts with solubilized cADoR in a simple biophysical reaction, which exhibits negative cooperativity between adenosine agonists and antagonists. In summary, [125I]HPIA bound saturably and reversibly to the solubilized cADoR via an apparent simple biophysical reaction. Both the pharmacological properties of the cADoR and the ability to interact with guanyl nucleotide binding protein(s) are maintained in this detergent system. Supported by a grant from the Oregon Affiliate of the American Heart Association, Inc. is a H.A.B. Dunning Memorial Fellow of the American Foundation for Pharmaceutical Education.


The neural elements associated with meningeal vasculature are similar to those of the cerebral vessels at the base of the brain. We are studying the relationship of nerve fibers associated with dural vasculature as a model to gain an understanding of the mechanisms which govern cerebral blood flow and cephalepia. Because a prominent perivascular mast cell population is associated with the dura mater, this tissue has been chosen for this investigation. The purpose of this study was to: 1. examine the sympathetic innervation of the rat meninges in normal animals and those in which the superior cervical ganglion (SCG) has been bilaterally removed; 2. test the hypothesis that the sympathetic nervous system is responsible for the generation of cephalepia; 3. determine the role of the sympathetic nervous system in the pathogenesis of cephalepia. The sympathetic nervous system is thought to play a role in the control of blood pressure and heart rate in this species. The purpose of this study was to: 1. examine the sympathetic innervation of the rat meninges in normal animals and those in which the superior cervical ganglion (SCG) has been bilaterally removed; 2. test the hypothesis that the sympathetic nervous system is responsible for the generation of cephalepia; 3. determine the role of the sympathetic nervous system in the pathogenesis of cephalepia. In our experiments, we have found that the sympathetic nervous system is responsible for the generation of cephalepia.
392.1 CENTRAL γ2 ADRENERGIC MECHANISMS IN THE RENAL NERVE MEDITATED PRAZOSIN AND DIURESIS PRODUCED BY ACUTE VOLUME EXPANSION. KP Patel, (SPON: SB Waller) Dept. of Physiol. & Pharm., University of South Dakota, Vermillion, SD 57069.

To determine whether central adrenergic mechanisms are involved in the natriuresis and diuresis produced by acute volume expansion (VE), urine flow (V) and sodium excretion (UNaV) from innervated and denervated kidney were measured before and after VE (1 ml/min for 20 min) in presence or absence of intracerebroventricular yohimbine (4 µg/kg/min) in inactively anesthetized Sprague Dawley rats. The innervated to denervated (I/D) ratio for V and UNaV indicated that VE caused a greater natriuresis and diuresis from the intact compared to the denervated kidney.

Data represent mean ± SEM, *p<0.05, No yohimbine vs. yohimbine. 

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<th>No Yohimbine</th>
<th>20 min</th>
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<td>Yohimbine UNaV</td>
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<td>0.24 ± 0.04 0.34 ± 0.07*</td>
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Data represent mean I/D ± SEM, *p<0.05, No yohimbine vs. yohimbine.

Supported by NIH HL-39046 and Parson's Fund, University of South Dakota.

392.3 INTRATHECAL CLONIDINE PRODUCES PRESSOR EFFECTS BY PERIPHERAL MECHANISMS, REDISTRIBUTION AND ACTIVATION OF VASCULAR ALPHAO ADRENOCEPTORS. G.F. Gehlhar, R.E. Solomon and R.K. Bhatnagar, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, IA 52242.

Intrathecal (i.t.) clonidine in rats produces, at lesser doses (1.0-10.0 µg), depressor and anticoagulative effects mediated by spinal α2 adrenoceptors and, at a greater dose (32.0 µg), pressor effects mediated by peripheral vascular α1- and α2-adrenoceptors (Soc. Neurosci. Abs., 13: 1246). The latter observation is further investigated by measuring randomicity in the blood, plasma, urine, and red cells after i.t. administration of [3H]clonidine. Concentrations of clonidine (in ng/mg) in spinal cord and brain after i.t. injection of 32.0 µg [3H]clonidine were:

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<th>Concentrations of clonidine (in ng/mg) in the blood were:</th>
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<td>0.21 ± 0.03 0.21 ± 0.03 0.21 ± 0.03 0.21 ± 0.03 0.21 ± 0.03</td>
<td>392.4</td>
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Data represent mean I/D ± SEM, *p<0.05, No yohimbine vs. yohimbine.

Supported by DA 02879, T32 OD 07069 and HL 38136.

392.5 RENAL CORTICAL AND MEDULLARY α2 ADRENOCEPTORS ARE UNREGULATED IN MALE SHR DIDS AND SHR-DO, E. Drouin-Iacocca and J.M. Woodworth, Dept. of Cell Biol. and Anat., Univ. of Alabama, Birmingham, AL 35294.

In male SHR, a spontaneously hypertensive rat (SHR-S), a high NaCl diet elevates arterial pressure by decreasing central sympathoinhibition. This study tests the hypothesis that renal α2 adrenoceptors in both cortex and medulla are upregulated following dietary NaCl loadings; an effect that may exacerbate hypertension in SHR-S. In SHR-S, the specific binding of the α2 adrenocorticosteroid (SR 141716A) in high affinity (10 nM) and low affinity (63 nM) is increased significantly 2 and 5 weeks after the initiation of a high (8%) compared to a basal, 1% NaCl diet. In contrast, during the established phase of NaCl exacerbated hypertension (10 weeks), there are no differences in renal [3H] pithyrol binding between diet groups. These experiments using SR 141716A, confirm the results of the high NaCl diet studies. The renal α2 adrenocorticosteroid (SR 141716A) in high affinity (10 nM) and low affinity (63 nM) is increased significantly 2 and 5 weeks after the initiation of a high (8%) compared to a basal, 1% NaCl diet. In contrast, during the established phase of NaCl exacerbated hypertension (10 weeks), there are no differences in renal [3H] pithyrol binding between diet groups. These experiments using SR 141716A, confirm the results of the high NaCl diet studies. The renal α2 adrenocorticosteroid (SR 141716A) in high affinity (10 nM) and low affinity (63 nM) is increased significantly 2 and 5 weeks after the initiation of a high (8%) compared to a basal, 1% NaCl diet. In contrast, during the established phase of NaCl exacerbated hypertension (10 weeks), there are no differences in renal [3H] pithyrol binding between diet groups. These experiments using SR 141716A, confirm the results of the high NaCl diet studies.

392.6 TACTILE STARTLE IN SPONTANEOUSLY HYPERTENSIVE (SHR), BLOODLINE HYPERTENSIVE (BHR), AND Wistar Kyoto (WKY) RATS IS UNAFFECTED BY GANGLIONIC BLOCKADE. C. H. Woodworth and A. K. Johnson. Departments of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Comparisons between SHR and WKY rats with respect to startle reactivity have yielded conflicting results. However, strain differences in startle reactivity may not be causally related to blood-pressure differences, since selective inbreeding can result in such traits being linked by chance in the present strain. Therefore, the startle (TS) was assessed on two occasions, 7-9 days apart, in male, 17-week-old SHR, WKY and BHR (the F1 progeny of SHR X WKY) with either ganglionic blocking agent or vehicle administered 20 min prior to the second test. Strain differences on TS were evident in the first (SHR>BHR=WKY), but not the second test. TS was significantly greater test-test decrease in TS than SHR or WKY, particularly at later trials and lower airpuff intensities. Hexemethonium (30 mg/kg, IA) produced a significant and equivalent drop in blood pressure across strains, but had no effect on TS. This suggests that startle reactivity is not sensitive to hypertensive changes produced by blocking sympathetic outflow to the periphery in these inbred strains.

Supported by NIH # RO1 HL33447 to A. K. Johnson.


Lesions of AHA chronically increase blood pressure in SHR-S but not in normotensive rats (NTR). The contribution of changes in baroreflexes to the hypertensive effect of AHA lesions, bilateral lesions of the AHA were made in 7-week-old SHR-S. Three days after the lesion group displayed significantly elevated systolic arterial pressure compared to the sham-operated group. At 10 weeks of age (3 weeks after the lesion was made), the lesion and control rats were instrumented with femoral, arterial and venous catheters, and 2 days later, the rats were volume loaded with whole blood and (5±6% of each rat's blood volume) 264 m/b/min. Systolic arterial pressure were 172 ± 4 and 165 ± 4 mm Hg (p<0.01) for the lesion and control rats respectively. Neither group displayed a significant blood pressure response to the volume load. In contrast, the lesion SHR-S displayed a significant elevation of heart rate (HR; 31 ± 12 bpm, initial HR 257 ± 9 bpm) following the load, whereas sham-operated SHR-S tended to slightly decrease heart rate following the load (12 ± 6 bpm, initial HR 356 ± 12 bpm). The results suggest that the AHA has an influence on baroreflex in SHR-S.

392.12 VASODILATORY EFFECT AND MECHANISMS OF CALCIUMIN GENE RELATED PEPTIDE (CGRP) IN RESISTANCE VESSELS FROM NONHYPERTENSIVE AND GENETICALLY HYPERTENSIVE RATS. S.-P. Han* and T. C. Westfall (SPOI: W. G. Wood). St. Louis University School of Medicine, St. Louis, MO 63104.

CGRP is known to exert cardiovascular effects. The current study was designed to investigate the effect and mechanisms of CGRP on resistance vessels from nonhypertensive and hypertensive rats. Isolated mesenteric arterial bed from Sprague-Dawley (SD) or from 14 weeks old spontaneously hypertensive rat (SHR) was perfused and superfused. Perfusion pressure was maintained at 80 mmHg. Arterial and venous catheters were chronically implanted and the animals' metabolic, thermal and cardiovascular responses were assessed. The independent effects of infusions of an "alpha" or "beta" agonist. Overall, the "alpha" agonist was non-thermogenic; whereas the "beta" agonist increased thermogenesis. The independent effects of the "alpha" or "beta" agonists potentiated the "beta"-thermogenic effect. This overall response profile, also observed in the cold acclimated animals, was enhanced in the cold acclimated rats. Conversely, renovascular hypertension suppressed thermogenic reactivity to "beta"-stimulation. The results suggest that the "alpha"-potentiation of "beta"-induced thermogenesis is not restricted to the cold acclimated endothelium and that the reduced reactivity of the hypertensive animal may be linked to its purported state of hyphydrothermism.
392.13 BRAIN STEM NORADRENERGIC TRANSMISSION IN PYRIDOXINE DEFICIENCY-INDUCED HYPERTENSION. M. Vissersath, C.S. Paulson, Y.L. Siew, and K. Dakhshinamurti. Dept. of Biochemistry, Fac. of Medicine, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

The synthesis of neurotransmitters dopamine and serotonin involves a pyridoxal-phosphate-dependent decarboxylation step. We have demonstrated previously that pyridoxine deficiency in adult rats is associated with dysautonomic and cardiac abnormalities leading to hypertension [Paulose et al. Hypertension 11(4) 1988]. The possibility that pyridoxine deficiency-induced hypertension results from altered noradrenergic transmission in the brain stem was investigated.

Adult male Sprague-Dawley rats (150-160 g) were fed either a pyridoxine-supplemented or pyridoxine-deficient diet for eight weeks. The calculation of potassium channels in the brain stem was studied using 3H]-methyl-alpha-ligand binding. There was a significant decrease in the potassium channels binding to the high and low affinity alpha-adrenergic receptors in the brain stems of pyridoxine-deficient rats compared with that in control rats. The increase in potassium channels was not accompanied by any change in binding affinity.

Pyridoxine deficiency also caused significant decrease in the maximal activity of dihydropyridine (DAP) deacetylase in the brain stem (27% of control). These changes correlated with data on sympathoadrenal turnover in the brain stem of pyridoxine-deficient rats. Our findings suggest that hypertension induced by pyridoxine deficiency may result from reduced alpha-adrenergic output in the brain stem. (Supported by grants from Manitoba Heart Foundation and MRC of Canada)


Orthostatic hypotension in autonomic failure is due to defective norepinephrine release and exaggerated renal sodium loss, especially at night during recumbency. The exact mechanism responsible for this deficit is not known. Atrial natriuretic peptide (ANP) is a recently discovered hormone that causes natriuresis and relaxation of smooth muscle. To determine the role of ANP in the renal sodium wasting of patients with autonomic failure was measured in patients with AF and in age-matched control subjects. All subjects were in sodium balance. Blood samples were collected during a 24-hr period at 4 hourly intervals with the patients recumbent.

The mean daily plasma concentration of ANP in patients with autonomic failure was 45 pg/ml and in controls 57 pg/ml (mean±SEM). Control subjects showed significantly higher levels of ANP during the night than during the day. In contrast, patients with autonomic failure exhibited no significant variation in the concentration of ANP through the day. ANP, however, responded appropriately to changes in extracellular fluid volume induced by the administration or withdrawal of fluidoside in patients with autonomic failure.

Since ANP is not increased in autonomic failure, it is unlikely that abnormalities in its secretion are responsible for the salt wasting and low blood pressure seen in these patients. Whether the blunted circadian rhythm of ANP secretion is primary or secondary to the autonomic failure remains to be elucidated.

Supported in part by NIH Grant NS 11631 and R-717.


Lowering blood pressure (BP) with the vasodilator, hydralazine, causes a greater rise in plasma CA, esp. epinephrine (EPI), in SHRSP than in normotensive WKY (Howe et al. J Cardiovasc Pharmacol 8:1113, 1986). Since both CA contents increase, difference in concentration and neurolevels of plasma CA are greater in SHRSP than in WKY. It was of interest to see whether plasma CA responses to other forms of stress are enhanced in SHRSP. In this study, effects of psychosocial stress and hypoglycemia on plasma and urinary CA were examined in conscious, unrestrained adult rats.

Initial BP readings and blood samples were taken at rest via an indwelling arterial catheter. To induce hypoglycemia, the animals were ad libitum fed a control diet of medium for 4 days. Plasma EPI rose from 180 to 3600 and from 840 to 5200 pg/ml in WKY and SHRSP resp. The corresponding rise in plasma norepinephrine (NE) was less than twofold. Most of the rise in EPI had occurred in SHRSP by 10 min but was unchanged in WKY at that stage. Other rats were made hypoglycemic by i.v. administration of insulin and somatostatin. Results appear in the Table.

<table>
<thead>
<tr>
<th>Treatment Time</th>
<th>WKY</th>
<th>SHRSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Plasma NE (pg/ml)</td>
<td>140±30</td>
<td>1190±350</td>
</tr>
<tr>
<td>Plasma EPI (pg/ml)</td>
<td>10±0</td>
<td>1450±3250</td>
</tr>
<tr>
<td>Plasma Glucose (mg/dl)</td>
<td>100±200</td>
<td>1540±3700</td>
</tr>
</tbody>
</table>

The results confirm that the resting level of plasma EPI is substantially higher in SHRSP than WKY and that EPI is released preferentially in response to both metabolic as well as circulatory stress. They suggest that adrenal medullary function may be selectively augmented in SHRSP.


We have examined Neuropeptide Y (NPY) production in primary atrial myocyte cultures from neonatal rats. NPY content was measured in cell extracts and in culture medium by gel filtration followed by radioimmunoassay. Exogenous NPY was stable in the culture medium for at least 48 hours. NPY mRNA in cell extracts was quantitated by Northern analysis.

Atrial myocyte dissociated cell cultures were maintained in complete serum free medium for up to 3 weeks. NPY-immunoreactivity was identified in atrial myocytes in cultures using the avidin-biotin/peroxidase complex method. Spent culture medium collected over 48 hours contained 0.9 pmol NPY/atrial equivalent. NPY content was reduced 5-fold when cultures were grown in medium containing 100 mM dexamethasone or 100 μM dexamethasone and increased 2-fold by growth in medium containing 100 mM phorbol myristate acetate (PMA). NPY mRNA by Northern analysis showed similar changes in response to these treatments.

Thus cultured rat atrial myocytes produce and secrete NPY. The production of NPY can be regulated in culture by distinct second messenger systems. Experiments are underway to examine further the regulation of NPY production in atrial myocytes. Support: NS0168, DA-00266, DA-00097.
393.1

Chronic benzodiazepine administration has been reported to decrease binding and function at the GABA-A receptor complex in vivo and in vitro. To assess the effects of chronic benzodiazepines in a controlled setting, we administered clonazepam, 0.1, 1, and 10 µM, to chick cerebral neurons in primary culture. GABA-A receptor function was assessed by muscimol-stimulated uptake of [3H]GABA as described by Thampe and Barnes (J. Biol. Chem., 259:1753, 1984). All assays were performed after 10 days of culture. Chloride uptake in cells treated with vehicle alone for 10 days was similar to controls. Uptake after clonazepam, 1 µM, for 2 and 4 days was similar to controls, and there was a trend toward increased uptake after 6 days. After 10 days, uptake was markedly decreased at all doses of clonazepam evaluated. Chloride uptake was also unchanged compared to controls after 2 days of clonazepam, 0.1 and 10 µM, but in both cases was substantially decreased at 10 days. These results in cultured neurons corroborate reports of decreased GABA-A receptor function in animals, and indicate that downregulation in cultured cells occurs at physiologically relevant drug concentrations.

393.3
DIFERENTIAL EFFECTS OF ZINC ON VERTEBRATE NEURAL GABA RECEPTORS. T.G. Sharp* and A. Cornish* (SPON: M. S. Reif). MRC Neuropharmacology Res. Grp., Dept. of Pharmacology, London University, School of Pharmacy, London U.K.

Zinc is an abundant ion in the mammalian central nervous system where it is concentrated into discrete areas including the hippocampus and the cortex. On cultured rat superior cervical ganglion neurones using patch clamping techniques, 10-100µM zinc resulted in a reversible inhibition of GABA-evoked membrane current responses. This inhibition was more potent in neurones derived from foetal rats compared to cultures prepared from post-natal and adult animals. However using intact, chick cerebral neurons in primary culture, K+d 21.7% ss 18.3% ss 15.3% ss 13.3% ss s= p<.005; paired t-test. The GABA enhancement of [3H]PCTZ binding was reduced by 67% (p<0.02) in neocortical membranes from Ro 15-1788 treated rats, as compared to the control group. Thus, chronic exposure to a BDZ receptor antagonist, Ro 15-1788, has a prolonged effect on the coupling between the BDZ and GABA components of the receptor complex. Supported by USAF grant AFOSR 87-0364 to T.J.M.

393.4

Earlier, we reported that microtoxin and several "cage" convulsants potentiated the protective effect of NaCl against heat inactivation (60°C, 30 min.) of [3H]Flunitrazepam binding sites on rat brain membranes. We now report that a series of polychlorinated convulsants, all of which potently inhibit the binding of [35S]-TBPS to rat brain membranes, also potentiate the protective effect of NaCl against heat inactivation of [35S]-TBPS binding sites with a rank-order of potencies which is similar (but not identical) to the rank-order of potencies for [35S]-TBPS binding. The most potent protector was α-endoosulfolan (25%, 2.6 µM) followed by eserin (12%, 0.2 µM), hexachloroperoxide (24%, 0.96 µM), and dieldrin (25%, 1.3 µM) to toxaphene (35%, 1.3 µM) and lindane (25%, 3.1 µM). As inhibitors of [35S]-TBPS binding, the rank-order of potencies was as follows: α-endoosulfolan (25%, 11 µM), eserin (52%, 0.2 µM), dieldrin (130 µM), hexachloroperoxide (170 µM), toxaphene (185 µM) and lindane (210 µM). These results provide further confirmation of a direct molecular coupling of picrotoxin (TBPS) and benzodiazepine (flunitrazepam) binding sites in the GABA-A receptor complex.

393.6

Pharmacological differences between insect and vertebrate GABA-A receptors suggest that multiple subtypes of GABA-A receptors may exist. Insect-dependent chloride channel of housefly thorax have revealed additional differences between insect and vertebrate receptors.

Addition of sulfoPhyryl reducing agents to membrane preparations from housefly thorax increased binding of [35S]-TBPS, but had no effect on rat brain membranes. N-Ethylmaleimide treatment eliminated [35S]-TBPS binding to housefly membranes (150±10 µM) but did not alter binding to rat membranes. These results suggest sulfhydryl groups play a role in determining binding interactions at the insect TBPS site. Insect and vertebrate receptors also differed in their interactions with steroidal compounds which potentiate chloride channel opening. Among the steroids tested, 5α-androstane-3α,17β-diol was the most effective at potentiating binding of [35S]TBPS to insect receptors (IC50<10 nM) but had only a small effect at the vertebrate site (IC50>100 µM). These results demonstrate that steroids interact with a unique binding site on GABA-A receptors. We are currently assessing the effects of these steroidal agents, on GABA-A receptors of identified neurons from cockroach.
The major inhibitory neurotransmitter in the brain, γ-aminobutyric acid (GABA), acts at specific neuronal receptors. In addition to GABA, the receptor possesses benzodiazepine, a class of anxiolytics. This GABA-Benzodiazepine receptor complex is composed of α and β subunits to which the benzodiazepines and GABA bind, respectively. While the receptor is generally believed to have a uniform subunit composition throughout the brain, autoradiographic studies with GABA ligands suggest structural heterogeneity in the cerebellum.

To examine further the expression of the GABA/Benzodiazepine receptor in the bovine cerebellum, we have used in vivo hybridization histochemistry. Oligonucleotide probes complementary to the mRNAs encoding the α and β subunits of the bovine receptor (Schofield et al., Nature 228: 221, 1973) were chemically synthesized and labeled with 32P. Following hybridization and autoradiography, a uniform distribution of grains was detected over the granule cell layer with probes for both subunits. Intense labeling was also observed over the Purkinje and molecular cell layers with the α subunit probe. In contrast, no β subunit grains were detected in these layers. Our findings suggest that, as yet unidentified, β subunits exist. (Supported by NIH Grant MH42173 and the Mathers Foundation)
Amino Acids: GABA and Benzodiazepines VI

393.13

*GABA (γ-aminobutyric acid) and benzodiazepine ligands photofunctionalize α and β subunits.*

M. Bureau and R.H. Olsen (SFOM: D.J. Jenden), Department of Pharmacology, School of Medicine, and Brain Research Institute, University of California, Los Angeles, CA 90024.

GABA is an inhibitory neurotransmitter in the central nervous system. In many CNS regions the highest GABA concentration is present in the deeper layers of the cerebral cortex. The GABA receptor complex (GBRC) is composed of a putative protein component of peripheral-type benzodiazepine recognition sites (PBR) and an agonist, the 3H-muscimol. This complex is thought to be involved in the regulation of GABAergic neurotransmission.

In this study, we isolated a clone of PBR from rat striatum that hybridizes with a GABA receptor cDNA. The clone was used to isolate a cDNA clone for PBR from rat brain. The cDNA was then used to make a probe for a northern blot analysis of GABA receptor mRNAs in various regions of the rat brain.

The results of the northern blot analysis showed that the PBR is expressed in all regions of the rat brain, with the highest levels in the cerebral cortex and hippocampus. The PBR is also expressed in the substantia nigra and the amygdala, which are regions of the brain involved in the regulation of GABAergic neurotransmission.

The PBR is a ligand for benzodiazepine drugs, which are used to treat various neurological disorders. The results of this study suggest that the PBR may be a target for the development of new drugs for the treatment of neurological disorders.

393.14

**Effects of cocaine on benzodiazepine receptor labeling in vivo.**

N. E. Goodman and M. A. McLellan

Department of Pharmacology & Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130.

The chronic administration of cocaine has been reported to differentially affect a variety of neuronal systems including benzodiazepine receptor densities measured in vitro in several regions of the brain (Goodman et al., Soc. Neurosci. Abst., 13:724, 1987). This investigation was therefore designed to determine the effects of cocaine on the benzodiazepine receptors using in vivo labeling procedures.

Adult male rats were injected via chronically implanted jugular catheters with 25 μCi [3H]Ro 15-1788 5 min following pretreatment with cocaine (5, 10, 20 or 40 mg/kg, ip) or saline (1 ml/kg). The rats were sacrificed by decapitation 10 min following the intravenous injection. The brains were rapidly removed and dissected into cerebral cortex, hippocampus, striatum, cerebellum, thalamus and brain stem, and radioactivity was directly determined in each tissue sample using liquid scintillation spectrophotometry.

Blank values were determined by pretreating the animals with clonazepam (5 mg/kg, ip) 30 min prior to the intravenous injection. The results of this study will be discussed in relation to those obtained in vitro.

[Supported in part by USPHS grant DA04293.

393.15

**Isolation of cDNAs closely related to GABA-A receptor subunit polypeptides.**


Departments of Biology and Pharmacology, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, CA 90024-1606.

We address the molecular composition and heterogeneity of the GABA-A receptor. We have prepared oligonucleotide probes (derived from the sequences of Schofield et al., Nature 328:221-227, 1987) for GABA-A receptor subunits. These were used to isolate cDNA clones for GABA-A receptor polypeptides.

We isolated several independent clones that hybridize with alpha subunit sequences. These were part of a cDNA library in lambda-gt11 derived from bovine cerebral cortex mRNA by Dr. Rachel Neve. They have 70-80% identity with the published bovine alpha cDNA sequence.

Under hybridization conditions of medium stringency, these subunit specific monoclonal antibodies and cDNA probes will be used to isolate candidate cDNAs for GABA-A receptor polypeptides.

This work was supported by grants to AJT from NINCDS (NS22276 and NS22356), the Scottish Rite Schizophrenia Research Program and a program project grant to Dr. A.V. Delgado-Escueta (NS21908). AJM was supported in part by a Canadian MRC Fellowship and by a Fellowship from the Fondation de l'Industrie Pharmaceutique pour la Recherche.

393.16

**Purification of a mitochondrial benzodiazepine binding site protein.**


Fondation de l'Industrie Pharmaceutique pour la Recherche. Paris, France.

In the rat brain, the mitochondrial benzodiazepine binding site is localized to the inner mitochondrial membrane and has been shown to be differentially affected by drugs that act on the GABA-A receptor complex. In this study, we isolated a protein that binds to [3H]Ro 15-1788, a benzodiazepine ligand, and purified it to homogeneity.

The protein was purified using a combination of affinity chromatography and ion exchange chromatography. The purified protein was then used to study its interaction with [3H]Ro 15-1788 and to characterize its binding properties.

The purified protein was found to bind to [3H]Ro 15-1788 with high affinity (Kd = 30 nM). The binding was specific for benzodiazepine ligands and was not affected by the addition of GABA, which is a negative allosteric modulator of the GABA-A receptor complex.

The purified protein was also found to be sensitive to the effects of various drugs that act on the GABA-A receptor complex. For example, the addition of clonazepam, a benzodiazepine antagonist, decreased the binding of [3H]Ro 15-1788 to the purified protein.

The results of this study suggest that the purified protein may have therapeutic potential in the treatment of neurological disorders.

393.17

**A molecular characterization of the γ-aminobutyric acid (GABA) receptor protein and mRNA from rat hippocampus: Development.**

P. Gallois, N. Sato, R. Duman, and J. Talmiein.

Abraham Ribciff Research Facilities, Dept. of Pharmacology, Yeshiva University, New York.

GABA is a major inhibitory neurotransmitter in the CNS. The GABA receptor complex (GBRC) is composed of a putative protein component of peripheral-type benzodiazepine recognition sites (PBR) and an agonist, the 3H-muscimol. This complex is thought to be involved in the regulation of GABAergic neurotransmission.

In this study, we isolated a clone of PBR from rat striatum that hybridizes with a GABA receptor cDNA. The clone was used to isolate a cDNA clone for PBR from rat brain. The cDNA was then used to make a probe for a northern blot analysis of GABA receptor mRNAs in various regions of the rat brain.

The results of the northern blot analysis showed that the PBR is expressed in all regions of the rat brain, with the highest levels in the cerebral cortex and hippocampus. The PBR is also expressed in the substantia nigra and the amygdala, which are regions of the brain involved in the regulation of GABAergic neurotransmission.

The PBR is a ligand for benzodiazepine drugs, which are used to treat various neurological disorders. The results of this study suggest that the PBR may be a target for the development of new drugs for the treatment of neurological disorders.

393.18

**An in-vitro study of the effect of unsaturated fatty acids on the GABA/Benzodiazepine receptor complex.**

M.R. Witt and M. Nielsen, Psychopharmacological Research Lab., St. Hans Hospital, DK-4000 Roskilde, Denmark.

In our search for endogenous modulators of the GABA/benzodiazepine receptor complex (GBRC), we isolated oleic, erucic acid and docosahexaenoic acids from pig brain. At concentrations of 10-4 to 10-5 M, these compounds enhance the specific binding of benzodiazepine receptor agonists to rat membranes in-vitro via an increase in affinity (Kd effect).

The stimulation of the binding of benzodiazepine receptor agonists is additional to the binding enhancement of these compounds in vivo. The binding of agonist ([3H]Ro-151788) and inverse agonist ([3H]DMCM) is only slightly stimulated. The number of binding sites labeled by [3H]muscimol is increased 2-3 times (Bmax effect), while the binding of [3H]SRS-95333 is unchanged.

 Pretreatment of the membranes with A23187 or forskolin abolishes these effects of the fatty acids on the GBRC. The binding of [3H]Ro 15-1788 is decreased in a concentration-dependent manner. Whether unsaturated fatty acids have any physiological relevance in the regulation of GBRC is unknown.
A POSSIBLE ROLE FOR PHOSPHORYLATION IN THE MAINTENANCE OF GABA-RECEPTOR FUNCTION

Department of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203.

Using whole-cell recording methods we have observed a time-dependent decline in the responsiveness of cholinergic nerve terminals to the neurotransmitter GABA. In the present study we have investigated the role of ATP in this phenomenon. Cells were voltage-clamped at their resting potential (-55 to -70mV) and responses to pressure applied GABA were obtained at 10 minute intervals. Intracellular Ca++ was buffered with EGTA (11mM). The peak amplitude of the inward current evoked by 3µM GABA (~2nA) progressively declined to approximately 30% of its initial value after 30 minutes. Similar results were obtained when these two concentrations of GABA were applied in sequence to the same cell. "Run-down" of the response to 30µM GABA was reduced when 5mM Mg-ATP was present in the pipet solution (approximately 70% of initial current remaining after 30 minutes). Inclusion of 5 or 28mM ATP, an analog that donates a thiophosphate group resistant to hydrolysis, effectively prevented run-down. These results suggest that an ATP-dependent process, possibly phosphorylation, is involved in the functioning of the GABA receptor.

PEPTIDES: RECEPTORS IV


Neuropetide Y (NPY) can inhibit the nictoionic receptor mediated release of catecholamines from bovine chromaffin cells (Biglisch et al., J. Physiol. 291: 468 (1979)) suggesting the existence of specific NPY binding sites. Bovine adrenal medullae membranes (P3 fraction, 0.4-0.6g/ml) were incubated for 30 min at 37°C in HCl, pH 7.4, containing 0.005 M CaCl2, 0.005 M MgCl2 and 1 M HSA, in the presence of 125I-NPY. Binding was terminated by rapid centrifugation. Specific binding was determined from the difference between 125I-NPY bound in the presence and absence of 1 x 10-6 M unlabeled NPY. Binding was linear over a protein concentration range of 0.1 to 0.8 mg/ml. 125I-NPY was stable for 2 h under these conditions. The binding was saturable and reached equilibrium within 10 min at 0°C. Scatchard analysis of specific 125I-NPY binding using the LIGAND computer program indicated a best fit for a two site model with kD of 2.56 x 10-10 and 1.64 x 10-7 M and Bmax of 1.2 x 10-11 and 6.0 x 10-9 moles/mg protein, respectively. The t1/2 for dissociation was 9 min. Displacement curves for NPY-free acid, peptide YY, ovine or human pancreatic polypeptide revealed IC50's greater than 300 x 10-9 M for these structurally related compounds. Intact chromaffin cells bind NPY and have a Kd similar to that found for the high affinity site obtained from P3 membranes. (Work supported by American Heart Assoc., Inc.)

CHARACTERIZATION OF MULTIPLE NEUROPETIDE Y (NPY) RECEPTOR BINDING SITES FROM RAT BRAIN. Mary W. Walker and Richard J. Miller (SPON: S.P. Grossman) Univ. of Chicago, Chicago, Ill 60637.

Using mono-iodinated neuropetide Y (NPY), we observed high, moderate and low affinity receptor populations in rat brain. Only the high and moderate affinity sites were clearly detected in equilibrium studies. The majority of binding was to the moderate affinity population, approximately 50% of which was "lost" in the presence of 300nM NPY (Martel et al. Peptides 7: 55, 1986) suggesting the existence of specific NPY binding sites. In rat brain, NPY has high, moderate and low affinity receptor populations in dorsal root ganglion cells, olfactory bulb, septum, hippocampus and substantia nigra pons compacta and lateralis, area postrema, striatal terminalis and fimbria hippocampi. Moderate densities of 125I-NPY binding sites were seen in outer layers of the cortex, ventral pallidum, claustrum, striatum, thalamus, hypothalamic and amygdaloid nuclei, ventral tegmental area, central gray, reticular formation and inferior olive. Overall, the autoradiographic distribution of 125I-NPY binding sites resembles that of 125I-Bolton-Hunter NPY binding sites described earlier (Supported by MRC).
Thus, this pseudopeptide may serve as a pure BN dose of 0.5 ug. At the dose of 10 ug, it acts with IC_{50} value of 100 nM. In contrast, min at 25°C in the kinetic experiments.

Specific bindings in WB, SC and NT were linear between 0.2-2.08 tissue weight and reached equilibrium within 30 min at 25°C in the kinetic experiments. 

\( \text{H-BK binding (<1.0 nM) was saturable and indicated the presence of a high affinity site in these tissues. In competition experiments, D-\text{H-BK}(B_1 \text{ antagonist}) inhibited H-BK binding (IC_{50}=60 nM) while des-Am\text{Y}(Leu)^{14}-BKN was not affected at 1.0 ug. These studies indicated the presence of BK receptors in the guinea-pig WB, SC and NT.} \)

**494.7**

DIFFERENT DISTRIBUTIONS OF EPIDERMAL GROWTH FACTOR-LIKE IMMUNOREACTIVITY (EPIFAC) AND IMMUNOREACTIVE EPIDERMAL GROWTH FACTOR RECEPTOR (IR-EGF-R) IN RAT SPINAL CORD NEURONS

F. V. Joshi*, Y. Askarzadeh, E. K. Engel, USC Neurosurgical Center, Los Angeles, CA 90033

Distribution patterns of G_{L}-IR and IR-EGF-R in adult rat (Sprague-Dawley) spinal cord sections were compared using peroxidase-antiperoxidase immunocytochemical localization. EGF-IR was observed in the cytoplasm of neurons of ventral, intermediate, and dorsal horns but to a different apparent degree of staining intensity. Neuronal of some neurons also showed EGF-IR. The distribution of IR-EGF-R was distinct in different spinal cord regions. There is IR in the small dorsal-horn neurons. In the ventral horn there was intense cytoplasmic staining of the larger neurons (>20 um) and lesser staining of the smaller neurons (<20 um) in laminae.

Speculatively, EGF might be a transported-exported trophic peptide interacting with post-synaptic EGF receptors in the spinal cord. As suggested by the differential distribution of EGF and its receptors in this study, EGF synthesized in sensory neurons of the dorsal-horn may interact with EGF receptors on large motor neurons. Thus, failure of endogenous spinal EGF, could affect motor more than sensory neurons, and this might be relevant to diseases of preferential motor neuron degeneration.

**494.9**

ANTAGONISM OF CENTRAL BOMBESIN (BN) RECEPTORS BY (Psii3,14, Leu^{14})BN, Z. Merali1, T.W. Moody2 and O.H. Cov3.


This study assessed the ability of (Psii3,14, Leu^{14})BN to serve as a central BN antagonist. The i.v.villourogastrointestinal binding study in fresh frozen cortical rat brain slices revealed that (Psii3,14, Leu^{14})BN inhibited specific (1,000 uCi) BN binding activity with IC_{50} value of 100 nM. In contrast, (Psii10,14, Leu^{14})BN and (Psii12,13, Leu^{14})BN were less potent with IC_{50} values of 1000 and 3000 nM respectively. These results were obtained on rats on a regimen of 7.5 hr of food deprivation, followed by i.c.v. administration of (Psii3,14, Leu^{14})BN (20 ug) and/or 5-HT (50 ug) or CCK-8 (100 ng) in the lateral ventricle and the external capsule of the brain. BN antagonized these two effects, starting at a dose of 0.3 ug. At the dose of 10 ug, it completely blocked the effect of BN on food intake but only partially blocked its effects on grooming. The antagonist alone failed to alter behavior. Thus this pseudo peptide may serve as a pure BN antagonist in the rat CNS, with slightly better in vivo potency than the earlier compounds.

**494.6**

BINDING OF THE GROWTH HORMONE RELEASING PEPTIDE SK&F 110679 TO CENTRAL TRH AND GnrH HORMONE RECEPTOR systems.

S. E. Codd and R. F. Walker, Smithkline and French Labs., P.O. Box 1539, king of Prussia, PA 19406

SK&F 110679 (Bis-D-Trp-Ala-Trp-D-Phe-Lys-NH2) is an enkephalin-derived hexapeptide that specifically releases growth hormone in a wide variety of vivo and in vitro. Previous binding studies, using ligands specific for mu and delta opioid binding sites, demonstrated an inverse relationship between the opioid binding potency and the growth hormone releasing effectiveness of a series of SK&F 110679-related peptides (Codd and Walker, Neuropharmacology, 1988). In an effort to better understand its mode of action, we established a binding assay for the peptide using a ligand which had been tritium labeled at the histidine residue. A specific binding site was determined in the presence or absence of unlabeled BK. Specific bindings in WB, SC and NT were linear between 0.2-2.08 tissue weight and reached equilibrium within 30 min at 25°C in the kinetic experiments. 

Thus, failure of endogenous spinal EGF, could affect motor more than sensory neurons, and this might be relevant to diseases of preferential motor neuron degeneration.
394.11 CALIBRATION OF AMERSHAM HYPERFILM B-8 MAX(TM) (HBF) PLATES FOR QUANTITATIVE AUTORADIOGRAPHY (QAR) WITH [125I] Dense G. Baskin and Thomas H. Wimpy* (SPON: James W. Linte). Departments of Medicine and Pharmacology, University of Washington, Seattle, WA 98195, and V. A. Medical Center, Seattle, WA 98108.

HBF produces high contrast autoradiographic images with [125I] peptide peroxidase. The purpose of this film is [14C] peptide standards in terms of tissue-equivalent concentrations of [125I]. Plastic sections with standard concentrations of [125I] (Amersham "Microscales") and [14C] standards (which are expressed by the pure glial scar formed following transection for several neurotransmitters in vitro. In this report, we use quantitative receptor autoradiography to determine in vivo which receptor binding sites are expressed following transection of the optic nerve in the albino rabbit. After unilateral optic nerve transection, the rabbits were allowed to survive for 40–99 days. The brains were removed, frozen to −70°C, and sectioned (30 µm). The sections were then processed for quantitative receptor autoradiography with either radiolabeled bombesin, calcitonin gene related peptide, galanin, glutamate, somatostatin, substance P (SP) and vasoactive intestinal peptide.

394.13 IN-SITU DEMONSTRATION THAT SUBSTANCE P RECEPTORS ARE EXPOSED TO THE PROCESS OF GLIATION AFTER OPTIC NERVE INJURY. M. Zimmerman, T.S. Gaters, C.G. Boehmer*, D.J. Josspecia and P.W. Mantyh. Center for Ulcer Research and Education; Brain Research Institute; UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies have shown that glia can express receptors for various neurotransmitters in vitro. In this report, we use quantitative receptor autoradiography to determine in situ which receptor binding sites are expressed following transection of the optic nerve in the albino rabbit. After unilateral optic nerve transection, the rabbits were allowed to survive for 40–99 days. The brains were removed, frozen to −70°C, and sectioned (30 µm). The sections were then processed for quantitative receptor autoradiography with either radiolabeled bombesin, calcitonin gene related peptide, galanin, glutamate, somatostatin, substance P (SP) and vasoactive intestinal peptide.

394.14 EFFECTS OF INTRATHecal NEUROKININ RECEPTOR AGONISTS ON THE CARDIOVASCULAR SYSTEM OF THE CONSCIOUS FREELY MOVING RAT. E. Massey, T. Schauen, and C. Couture*. Dept. of Physiology, Faculty of Medicine, University of Montréal, Montréal, Québec, Canada.

This study aims to characterize the neuropeptide (NK) receptor which mediates the cardiovascular responses elicited by the intrathecal (i.th.) administration of SP. In addition to SP, neuropeptide A (NMA), and neuropeptide B (NKB), five NK receptor subtype selective agonists: [Pro9, Met(O2)11]SP, (A); 394.15 SYMPAThIC NEURONS EXPRESS HIGH LEVELS OF RECEPTORS FOR SENSORY NEUROPEPTIDES F. Gates*, R.J. Amsden, C.G. Boehmer*, K.C. William. N. Vigna, J.E. Maggio, and P.W. Mantyh. Brain Res. Inst., UCLA, LA, CA 90024, Dept. Biol. Chem. and Pharmac., Los Angeles, CA 90024, Dept. of Phys., Duke Univ. Med. Center, 27710.

To define which sensory neurotransmitters are involved in regulating post-ganglionic sympathetic neurons we used quantitative receptor autoradiography to determine which neuropeptide receptor binding sites are expressed by neurons in the rat and rabbir superior cervical ganglion and rabbit superior mesenteric ganglion. The neuropeptides examined included bombesin, calcitonin gene related peptide, galanin, somatostatin, substance K, substance P and vasoactive intestinal peptide. High levels of receptor binding sites for cholecystokinin, galanin, somatostatin, substance P and vasoactive intestinal peptide were present in all sympathetic ganglia examined whereas specific receptor binding sites for bombesin, calcitonin gene related peptide and substance K were not detected. These results suggest that sensory neurons use specific neuropeptides to modulate sympathetic activity.

394.16 FUNCTIONAL ROLE OF HIGH AFFINITY NEUROTENSIN (NT) BINDING SITES IN RAT BRAIN. S. Hashoff, M. Heutelburg, E. Müller, M. F. Patriz, M. Schuster, G. Schillinger and A. Vocke*, Pharm. Research, CIBA-GEIGY, Basel, CH-4002 Switzerland.

High and low affinity NT binding sites were characterized with [125I]Tyr-Gly-Phe-Arg-NH2 (Tyr-Arg) and [125I]NleMet 12-13 (NleMet) binding assays. Tyr-Arg binding was displaced by 80% with levo-lobastine (L), a marker of low affinity NT sites. NT and a synthetic pentapeptide analog (P) had IC50's of 27 and 43000 nM. In [125I]NleMet binding, I exerted a maximum of 70% inhibition, in contrast to NT (IC50's of 2.5 µM and 175 nM). P showed a Hill coef. of 0.6, indicating a clear-cut interaction with two sites. In contrast, there was competition with IC50's of 2.3 µM and 458 nM. These data confirm that [125I]Tyr-Arg binds to high and low affinity NT sites. They show also that L is specific for the low, and P strongly selective for the high affinity NT sites. In addition, TMR-labeled neuronal sites of the spinal cord in (silicon) at 3 m (maximum; 0.5 µM). L also tended to excite these neurons, but the shape was atypic, the concentration very high (3 µM) and the solution uncleavable (UDSC). In vivo, P produced a dose-dependent hypothermia after iv injection (first effect at 0.1 µg/rat), whereas L was inactive at the highest usable dose (70 µg/rat). In conclusion, P diminishes the functional role of NT, being 3–5 times more potent, whereas L was rather inactive. According to the selectivity of P for high affinity NT binding sites, these sites play a functional role.

394.17

THE CALCITONIN RECEPTOR. B.-L. Dana, Dept. of Medicine, Univ. of Calif., San Diego. La Jolla, CA 92039.

Specific, high-affinity (Kd<1 nM) cell membrane receptors for calcitonin and calcitonin-related peptide (CRP) were distributed throughout the central and peripheral nervous systems and also on some somatic tissues. Radiolabeled salmon calcitonin was used to study the binding to receptors on isolated membranes from: neonatal rat brain, adult rat brain, kidney, liver, 8 different cell lines, bovine kidney, human placenta. The highest levels of specific binding per mg of protein were found in brains from one week rat pups. Trichloroacetic acid precipitation revealed that the liver had the greatest amount of degradation activity of peptide. Cross-linking studies revealed that the neonatal rat brain receptor rohave a molecular weight of 60-70K. Current research involves the purification of receptor by affinity chromatography and electrophoresis, partial amino acid sequence analysis for the eventual isolation of the receptor cDNA with oligonucleotides.

394.18

MULTIPLE AFFINITY STATES OF BINDING SITES FOR (+)SKF10,047 AND 1-[(1-(2-THIENYL) CYCLOXYL]-PIPERDINE (TCP) IN RAT AND GUINEA PIG BRAIN MEMBRANES. G-Z. Zhou*, A.Katki*, P.Munson*, & D.Hodgson* (SPON: B.Cox), LTPB, RHIB, NIH, Bethesda, MD 20892.

We employed program "LIGAND" for simultaneous analysis of self- and cross- displacement studies and of "multiligand" dose-response surfaces, with or without 10-50 nM haloperidol (HAL). We find 3 classes of sites (Table). In guinea pig, we find 5 classes of sites: 2 selective for (+)SKF10,047 only one is suppressible with 10-50 nM HAL and 2 are TCP selective, HAL nonsuppressible sites (Table). Analysis of overlap indicated that the intrinsic thalamic neurons, the greatest amount of degredation activity of peptide. Cross-linking studies revealed that the neonatal rat brain receptor rohave a molecular weight of 60-70K. Current research involves the purification of receptor by affinity chromatography and electrophoresis, partial amino acid sequence analysis for the eventual isolation of the receptor cDNA with oligonucleotides.

394.19

QUANTITATIVE AUTORADIOGRAPHY OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) RECEPTORS IN RAT BRAIN: THE EFFECT OF FIXATION. M.G. King* T.H. Winyard* and D.G. Baskin. Deps. Biological Structure and Medicine, University of Washington, Seattle, WA 98195, and V.A. Med. Center, Seattle, WA 98168.

We evaluated the effect of paraformaldehyde (PAF) on the binding characteristics of IGF-I receptors in order to develop a method for localizing IGF-I receptors at the cellular level. Frozen sections (20 µm) from rat brains perfused with saline (control), 1% PAF, or 2% PAF were incubated in 0.05 nM 125I-IGF-I for 20 h at 5°C, with or without unlabeled IGF-I (0.25-50 nM) or related peptides (IGF-II and insulin), and assayed to Hyperfilm ß-Max (Amersham) for 3 days. The binding of IGF-I to 10 brain regions (including median eminence, CA3, and cerebral cortex) was quantified by computer densitometry of the autoradiographic images. The 3 groups (control, 1% PAF, 2% PAF) were not significantly different for specific binding (78±1.0%, 76±1.0%, 82±1.0%), binding specificity (IGF-I>IGF-II>insulin), and the reversibility of binding. The number (Bmax) and affinity (Kd) of IGF-I receptors of unfixed brain (2.0±0.2 pmol/mm², 2.41±0.2 nM) was not affected by 1% PAF (1.78±0.2 pmol/mm², 2.46±0.2 nM). 2% PAF increased the Bmax (3.71±0.5 pmol/mm²) and decreased the Kd (4.59±0.5 nM), but total specific binding capacity was unchanged. These findings indicate that tissue fixed in PAF is suitable for localization and characterization of IGF-I receptors. Supported by NIH Grant NS 24809 and the V.A.

395.1


The rat thalamus contains a dense plexus of met-enkephalin containing nerve fibers and terminals. The cell bodies that give rise to this plexus have not been identified. This study proposes a source for this enkephalinergic input since we have identified the intrinsic thalamic neurons, the adult rat brain, kidney, liver, 8 different cell lines, bovine kidney, human placenta. The highest levels of specific binding per mg of protein were found in brains from one week rat pups. Trichloroacetic acid precipitation revealed that the liver had the greatest amount of degradation activity of peptide. Cross-linking studies revealed that the neonatal rat brain receptor rohave a molecular weight of 60-70K. Current research involves the purification of receptor by affinity chromatography and electrophoresis, partial amino acid sequence analysis for the eventual isolation of the receptor cDNA with oligonucleotides.

395.2


Published immunohistochemical procedures vary widely in times of incubation/washes and the concentration of immunoreagents. We examined the application of the PAP localization system on rat brain 40μm vibratome sections fixed by perfusion with pH 8.5 2% formaldehyde/O.O5% glutaraldehyde for 20 h at 5°C, with or without unlabeled IGF-I (0.25-50 nM) or related peptides (IGF-II and insulin), and assayed to Hyperfilm ß-Max (Amersham) for 3 days. The binding of IGF-I to 10 brain regions (including median eminence, CA3, and cerebral cortex) was quantified by computer densitometry of the autoradiographic images. The 3 groups (control, 1% PAF, 2% PAF) were not significantly different for specific binding (78±1.0%, 76±1.0%, 82±1.0%), binding specificity (IGF-I>IGF-II>insulin), and the reversibility of binding. The number (Bmax) and affinity (Kd) of IGF-I receptors of unfixed brain (2.0±0.2 pmol/mm², 2.41±0.2 nM) was not affected by 1% PAF (1.78±0.2 pmol/mm², 2.46±0.2 nM). 2% PAF increased the Bmax (3.71±0.5 pmol/mm²) and decreased the Kd (4.59±0.5 nM), but total specific binding capacity was unchanged. These findings indicate that tissue fixed in PAF is suitable for localization and characterization of IGF-I receptors. Supported by NIH Grant NS 24809 and the V.A.

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PEPTIDES: ANATOMICAL LOCALIZATION III
These results demonstrate that retinal terminals are processed by the PAP method, postfixed in osmium tetroxide, perfused as above, and vibratome sections of LGN were mate immunoreactivity (NAAG-IR) in the retinal ganglion cell bodies, of the denervated layers, suggesting effects on food intake. We sought to identify other cell-to-cell interactions that may underlie their behavioral effects.

Formaldehyde fixed sections were double-labeled using a mouse monoclonal antibody (Ab) against CCX-B and a rabbit polyclonal Ab against ENK followed by incubation with anti-mouse (goat)-FITC and anti-rabbit (goat)-Texas Red. Mix-matching the secondary antibodies resulted in total abolition of immunostaining.

Accessory magnocellular cells were immunoreactive for CCX and ENK-containing fibers surrounded these neuronal groups with little penetration to deeper lying cells. Approximately 5% of immunolabeled ENK cells in the periventricular periventricular region also contained CCX. The dense periventricular division was innervated with fibers containing both peptides in common post-synaptic site. These findings suggest that CCX may interact with ENK by multiple mechanisms. Supported by NS12311 (SNK), R01-MH-05595 (PLF).

A previous report (Inagaki et al., Brain Res., 260, 143-146, '83) has suggested that the peptide neurotensin is contained in neurons of the pituitary cortex which project to the mediodorsal thalamic nucleus (MD) in young rats. To confirm this, we have examined the distribution of neurotensin-like immunoreactive (NTTR) fibers in MD using three antisera directed at different parts of the neurotensin molecule (Emson et al., J. Neurochem., 38, 992-999, '82). In adult MD, NTTR fibers are sparse almost completely at the medial edge of MD with a few, poorly-stained NTTR fibers in the central part of MD. In contrast, during the first postnatal week, both the medial and central portions of MD, densely stained with the NTTR antibody (AB) against N, showed marked differences in density and distribution of NTTR fibers in MD also occur. In seven day old rats, the medial and central portions of NTTR fibers are continuous, but by ten days a non-immunoreactive zone appears between them. This non-immunoreactive enlarges until the medial contingent of NTTR fibers reaches its adult position at the medial edge of MD.

Supported by NIS research grant NS09518.

We have previously identified N-acetyltyrosylglycine

N-acetylaspartylglutamate-immunoreactivity (NAG-IR) in the retinal ganglion cells (RGC) of the rat and in the neuropil of their targets. If NAGS serves as a transmitter for RGC, as we have suggested, then it should be found in their terminals. To determine whether these terminals contain NAGS, one adult cat was deeply anesthetized and one eye removed. The cat was reanesthetized 10 days later and perfused with PBS followed by 4% paraformaldehyde and 1% 3-(3-dimethylaminopropyl) carbodiimide in 0.1 M phosphate buffer. Frozen sections of LGN were processed for NAAG-IR by preincubation with 0.1 M phosphate buffer followed by incubation with anti-mouse (goat)-FITC and anti-rabbit (goat)-Texas Red. Mix-matching the secondary antibodies resulted in total abolition of immunostaining.

435.5

Supported by NIS research grant NS09518.

The transmitter γ-amino butyric acid (GABA) has been previously localized in rat at the light (LM) and electron microscopic (EM) levels. These studies suggest a widespread distribution of GABA neurons and terminals in the spinal cord dorsal and ventral horns. In the present study, GABA containing profiles are immunostained and visualized at the LM level in the monkey lumbar spinal cord. EM analysis of GABAergic neurons and terminal interactions are also reported. Four animals (Macaca fascicularis) were perfused with mixed aldehydes and the lumbar cord removed. The tissue was sectioned at 50µm on a vibratome and immunostained with anti-GABA using the PAP technique. The analysis demonstrated GABAergic cells concentrated in the superficial dorsal horn (SDH); however, there were present in all spinal laminae except lamina IV. Small diameter oval to round cells were located in the upper laminae while larger diameter cells with visible dendrites were present in deeper layers. Positive GABAergic profiles, presumably terminals, were scattered diffusely over the SDH, lamina X and around motoneurons in the ventral horn. EM analysis demonstrated both myelinated and unmyelinated GABAergic axons. GABAergic cell bodies with large immunoreactive nuclei were seen. GABAergic terminals contained mainly round clear vesicles and often a few dense core vesicles and synapsed onto labeled and unlabeled somata, dendrites and other axon terminals. (Supported by NS12755.)

435.6


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435.7


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435.8

CA2 (RESISTANT) SECTOR OF HUMAN HIPPOCAMPUS DEFINED BY CHROMOGRENIN-LIKE IMMUNOREACTIVITY. D.D. Murphy, D.H. George* and L. Kovalinski*, Dept. of Pathology, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada. 57N OMO.

Status epilepticus and cerebral anoxia can result in the destruction of most neurons, but characteristic sparing of a narrow sector, known as the resistant sector, that appropriately correspond to the CA2 sector defined by Golgi stains. Although certain enzymes show distribution gradients among the hippocampal sectors, there has been no reports of specific CA2 markers. Here we show that such sectors are defined by the perikaryal chromogrenin-like immunoreactivity of its pyramidal neurons. The monoclonal antibody (LK2H10) produced against a human choroid plexus secretory granulocyte (AM J Path 115: 458, 1984) recognizes the human chromogrenin A molecule as well as an array of polypeptides (chromogrenins) with Mr down to 28,000. A normal human hippocampal fixed for 24 hours in PFG fixative (Neuroscience 7:1779-1783, 1982) were Immunocytochemically stained with LK2H10 by the ABC method. In all cases there was intense staining of the mossy fibers, as well as granular cytoplasmic staining of all pyramidal neurons in a sector corresponding to CA2. Immunostaining of the mossy fibers to a sharp wedge-shaped lateral limit. No other neurons in the hippocampus proper were stained, although the density of a subset of neurons in the dentate gyrus and the hilum.
A PROPOSED MIGRATORY ROUTE FOR LUTINIZING HORMONE-RELEASING HORMONE (LHRH)-IMMUNOREACTIVE NEURONS FROM THE MEDIAL OFFA CTORY PLACOD E TO THE FOREBRAIN IN THE MOUSE. A STUDY USING TRITIATED THYMIDINE AND LHRH IMMUNOCYTOCHEMISTRY. Harlene Schwann-Pluda and Donald W. Pfaff. Rockefeller University, New York, N.Y. 10021.

Swiss mice (two in each age group) were injected with tritiated thymidine (5 uCi/gram of body weight) on day 11, 14, 16 or 18 of pregnancy. Fetal brains were collected 1 hour later and processed for LHRH immunocytochemistry and autoradiography. Autoradiographic silver grains were first detected in a few cells within the epithelium of the medial olfactory placode at 11 days of gestation. From days 14 to 18, most of LHRH cells were located in the placode, on the nasal septum in company with the nervous terminals and vomeronasal nerves, and in the ventromedial forebrain caudal to the olfactory bulb. Some of these cells were associated with the nervous terminals, but others (the majority) arched into the septal and preoptic areas of the developing brain. This finding, as well as relative numbers of immunolabeled cells at different ages, supports the notion of a migratory route. Observations of autoradiographic sections showed no thymidine labeling of LHRH cells: the cells of the placode appeared to have undergone division earlier. The nervous terminals, a derivative of the medial olfactory placode, may serve to guide at least one population of LHRH cells into the forebrain. Supported by NIH grant NS 19662 and funds from the Whitehall Foundation (N.S.-F.).


Tachykinins, identified by an antibody that recognized both SP and SK, and the binding sites for SP and SK are densely concentrated in the habenulo-interpeduncular system. Tachykinins are synthesized in the medioventral habenula and transported to the lateral and rostral subnuclei of the habenular complex. The habenular complex shows plasticity in response to lesions of the contralateral habenular projection. Intrinsic tachykinins-containing interneurons are present in the ventral division of the habenula. The SP and SK fibers of the habenular complex link the habenula and the nucleus accumbens. Studies on the spinal cord showed that the tachykinin-containing interneurons located in the spinal cord and the nucleus accumbens are present in the spinal cord and the nucleus accumbens. Supported by NIH grant NS05569.


CGRP-IR is localized in nerve fibers innervating the rostral hepatopatrical system. These fibers are abundant in the biliary pathway, whereas in the liver they are usually restricted to the interlobular space in association with the portal triad. The rich CGRP innervation is also associated with the vena portae. In this study we have investigated the effect of chemical and surgical denervation on the CGRP-IR fibers by examining the hepatopatrical system of the rat by means of immunohistochemistry and radioimmunoassay (RIA). Neuronal treatment with the sensory neurotoxin capsaicin causes a long-lasting reduction in the CGRP-IR fibers. These results suggest that the CGRP innervation of the hepatopatrical system mainly originates from extrinsic, sensory neurons which are likely located within spinal ganglia. Supported by NIH grant DK38752 and SKB Fellowship.


We studied the morphological distribution and number of neuropeptide Y-immunoreactive (NPY-IR) neurons in the rat corpus callosum in male and female Sprague-Dawley rats. Crystall sections (30-50 µm) cut in the coronal, sagittal and horizontal planes were processed by peroxidase-antiperoxidase immunocytochemistry. The corpus callosum in the rat was divided into an anterior and posterior portion using the anterior border of the fornix as the dividing landmark (2mm posterior to bregma). Comparisons were made between anterior and posterior portions of the corpus callosum, the right and left hemispheres, and between male and female rats. The number of NPY-IR neurons in the rat corpus callosum is small and medium-sized bipolar and multipolar neurons (7-15 µm in diameter). The highest concentration was observed in the fornix more than the corpus callosum. The anterior portion of the arm corpus callosum contained about twice the number of NPY-IR neurons as the posterior portion. There was no significant morphological, distributional or numerical difference between hemispheres nor between male and female rats. We are presently investigating NPY-IR neurons in the rat corpus callosum of cats and humans. The morphology and size of the NPY-IR neurons appear similar to those in the rat. The distribution of NPY-IR neurons in the human corpus callosum appears sparse.

IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF TACHYKININ-RELEASENING HORMONE PROPERTIES IN THE RAT BRAIN. N. Liao*, B. Culbertson, A. Sklar, C. U.C.R. & E. Schinco, UCLA Departments of Anatomy, Psychiatry and Brain Research Institute, Los Angeles, CA 90024.

In order to study the ultrastructural localization of pro-TNK, we have raised antibodies against a cytoplasmic fragment corresponding to the sequence d37-49 of the neurotensin precursor. Using preembedding immunostaining, we have investigated two groups of neurons already known to contain pro-TNK: one located in the hypothalamic paraventricular nucleus (PVN) and another one in the raphe magnus. In the positive neurons of HPN only dense core vesicles (80-100 nm in diameter) which were very numerous were labeled. Immunostained cells, dendrites and endings were frequently observed in contact with unidentified elements. In the median eminence, numerous free endings containing labeled dense core vesicles were detected. In the raphe nucleus a different pattern of labelling was observed. The positive neurons contained only a very few dense core vesicles (60-80 nm in diameter) which were all labeled, the most prominent labeled organelle being the Golgi apparatus. Stained cell bodies and dendrites were very frequently seen in contact with unlabeled endings. These results suggest that pro-TNK and/or fragments of pro-TNK could play a neuromodulator/neurotransmitter role in different brain areas and could also be released into the pituitary portal plexus. They also demonstrate that pro-TNK can be differentially processed by neurons which have different functions.


CCK-like immunoreactivity was localized in the brain of the little brown bat using light microscopic immunocytochemistry. Tissue was fixed in Bouin solution, embedded in paraffin, and sectioned. Immunoperoxidase-antiperoxidase immunocytochemistry was performed in three major pathological states: median forebrain bundle, habenular nucleus, and mammillary peduncle. Particularly dense terminal plexuses were formed in the medial septal and dorsal hippocampal areas, and in the basal amygdaloid nuclei, dorsomedial, parabrachial, and supramammillary nuclei, area postrema and nucleus tractus solitarius. Immunoreactive fibers were present in the olfactory, amygdaloid, interpeduncular, dorsal tegmental, and supramammillary nuclei, medial preoptic, and posterior hypothalamic areas, hippocampus and cerebral cortex.
PEPTIDES: ANATOMICAL LOCALIZATION

DISTRIBUTION AND NOVEL RESPONSE TO INJURY. E. E. Black

Cord of the molly, Poecilia latipinna, was investigated. Some fibers traversed the central nervous system (CNS). Further, injury to any preterminal vertebra or at the level of the terminal vertebra also resulted in complete disappearance of GAL-LIR in the spinal cord. These studies indicate that a process, containing a protein with bursicon-like activity. The localization of somatostatin immunoreactive (SS ir) perikarya and fibers in Agnathans. The largest population of SS ir fibers were observed in the dienecephalic system, followed by the telencephalon and mesencephalon, and a scant amount in the mesencephalon. In the mouse SS ir is present in the dienecephalic system but not the telencephalon or mesencephalon. In the brain of A. chrysogaster we have identified a group of preterminal fibers. These fibers were seen in optic tectum, torus semicircularis and interpeduncular nuclei. The nociceptive system, was also revealed in the olfactory bulb but not the olfactory system. Substance P (sP) like immunoreactivity (SPLI) in the olfactory epithelial layer is not the same as the somatostatin-like immunoreactivity (SS Li) in the olfactory epithelial layer. In contrast, application of colchicine (250) to the olfactory nerve for 24 hours produces no depletion of SPLI. Anti-somatostatin antisera labeled the primary olfactory projection in the olfactory bulb but not the olfactory system. No immunoreactivity was obtained in all these sites. Identical depletion is obtained (5 d) after application of 5-HT antagonist to the olfactory nerve. Substance P is a transmitter in the olfactory nerve and olfactory bulb. Substance P is also present in the olfactory nerve. Substance P, considered as a transmitter or modulator of the nociceptive system, was also revealed in the olfactory nerve. Substance P in the olfactory bulb of a mammal (Kream RM et al., JCN, 222:140, 1984) and recently in the primary olfactory projection of A. chrysogaster (Sasbo T et al., Neurosci.Lett., 81:245, 1987). Standard immunohistochemical treatment of paraffin sections with anti-substance P antisera (1/8000) shows an intense sP-like immunoreactivity (SPLI) in the olfactory epithelium, nerve and olfactory bulb as well as in several telencephalic and mesencephalic areas. The expression of substance P in the olfactory nerve causes a reduction (in 15 d) or complete depletion in 35 d, in all these sites. Identical depletion is obtained (5 d) after application of a 5-HT antagonist to the olfactory nerve for 15 min. In contrast, application of colchicine (250) to the olfactory nerve for 24 hours produces no depletion of SPLI. Anti-somatostatin antisera labeled the primary olfactory projection in the olfactory bulb but not in the olfactory system. Supported by the French Medical Research Foundation.
395.21
ULTRASTRUCTURAL LOCALIZATION OF SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE BRAIN OF LIMULUS POLYPHEMUS.

Light and electron microscope studies have shown the corpora pedunculata (C.P) in Limulus to be synthetically complex and morphologically diverse (Fahrenbacher, Tiss Cell. 9:157-166, 11:163-200). These anatomical studies have been complemented and extended with immunohistochemical methods in a survey of neurotransmitters in the Limulus brain including a positive reaction for substance P-like immunoreactivity (SPL) (Chamberlain and Engelbrecht, J. Comp. Neurol. 208:304-315). Here we report the first demonstration of SPL in the brain of Limulus at the ultrastructural level.

In the present study a monoclonal antibody against substance P raised in rat (Accurate Chemical, 11:163-200) was used to bind substance P-like antigenic sites. Visualization of labeled sites was effected by indirect enzymatic staining with the PAP/DAB procedure (Eldred et al., J. Histochim. Cytochim. 31:285-292). This technique was used to identify neuron types in the CP that had a substance P-like positive reaction. Results suggest only that the type A neurons display SPL in the Kenyon cell layer. In contrast, possible type B neurons display a weaker reaction in the peduncles and regions of Kenyon cell telodendra, often in close proximity to more heavily labeled type A neurons.

These results are an additional line of evidence suggesting the presence of substance P in Limulus. This technique allows the specific neuronal circuitry of substance P-like fibers to be elucidated from populations of neurons containing other neurotransmitters. Continued application of this technique should lead to a detailed understanding of the specific connectivity in the brain and lateral eye of Limulus at the EM level. Supported by EY03446 and EY06654.

395.23
AN ATRIAL NATRIURETIC-LIKE SUBSTANCE IN THE MARINE GASTROPOD APLYSIA. M. C. Castellucci and J. Gutkowska. Neurobiology Lab., IRCM Clinical Research Institute of Montreal, Montreal, CANADA, H2W 1R3.

The atrial natriuretic factor (ANF) is a member of a family of peptides that have been first isolated and purified from mammalian atria. It is also present in other peripheral and central nervous systems of vertebrates. Since ANF seems to be related to the water balance and cardiovascular system of an organism, we were curious to find if this peptide could be detected in Aplysia and what was its biological role.

We were able to detect ANF-like immunoreactivity in the hemolymph of Aplysia (5-10 pg/ml); the hemolymph extract had an HPLC profile similar to the ones obtained from vertebrates with a prohormone and a circulating hormone peak. Significant immunoreactivity was also detected in various organs, such as the heart, the aortae, the gametolytic gland, the salivary gland and the central nervous system. The immunoreactive ANF could be detected in radioactive assay procedure. Supported by N.R.C. MA-10047.

395.24
IMMUNOHISTOCHEMICAL STUDY ON VASOACTIVE INTESTINAL POLYPEPTIDE IN THE LIMULUS POLYPHEMUS. A. Soinila and G.J. Mptios. Hatfield Marine Science Center, Oregon State University, Newport, OR 97366.

Vasoactive intestinal polypeptide (VIP) is a putative neurotransmitter/neuromodulator in several vertebrate and invertebrate species. The present study was undertaken to define simple experimental models that would allow us to examine the role of VIP in the nervous systems of marine molluscs. To this purpose, immunohistochemistry in the Limulus was undertaken. Each buccal hemiappendage contained on the coelomic (caudal) surface a single 100 µM VIP neuron. A cluster of small VIP cells (30 µm) was observed constantly near the outlets of buccal roots 1 and 2 that are connected to the buccal muscles. Another group of small VIP cells was located ventrally to the commissure on each side. In Aplysia, but not in Pleurobranchaea, the buccal (rostral) surface contained a large number of VIP neurons which extended from the lateral 5-region to the central region of the ganglion.

These results provide a morphological basis for comparative studies on the effects of VIP on Aplysia and Pleurobranchaea feeding behavior. Of particular interest is to use such biochemical and neuroanatomical methods as probes to identify groups of neurons whose group dynamics can then be analyzed to determine the principles of self-organizing activity and of the role of variability in it (e.g. see Mptios et al., 1988, these proceedings, and Mptios, G.J., and Cohen, S. C. J. Neurobiol. 17: 517, 1986).

This study was supported by the grant APOS-86-0076 to G.J.M.

396.1
THE QUANTAL NATURE OF SYNAPTIC TRANSMISSION FROM PHOTOCEPTORS TO BIPOLAR CELLS. D.R. Maple and F.S. Werblin. Graduate Group in Neurobiology, University of California, Berkeley, CA 94720.

Bipolar cells of the Tiger Salamander retina were voltage clamped with patch electrodes in light adapted retinal slices. Transmission from photoreceptors was measured by the fact that bipolar cell transmission was turned off postsynaptically in light adapted slices. Relatively large light responses obtained in these cells suggest there is a cone transmitter that is not glutamate-like.

396.2
GLUTAMATE RESPONSES IN SOLITARY BIPOLAR CELLS FROM SALAMANDER RETINA. A.A. Hirao, J.A. Hincht, and P.R. MacLeish. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

We have studied the currents evoked by L-glutamate and D,L-APB in single isolated bipolar cells electrically dissociated from the adult tiger salamander retina. The cells were maintained in serum-free media at 37°C for up to several weeks before use, with no obvious change in response properties. During recording, the cells were superfused with a salt solution. In bipolar cells, identified by their characteristic morphology and I-V curve, glutamate-induced currents were recorded using whole-cell patch clamp recording techniques. The current responses strongly resembled those produced by this mechanism.

Electrodes had a tip diameter of about 0.1 µm and contained (in mM): D-aspartate, 100; NaCl, 120; NaH2PO4, 2.5; MgCl2, 2.5; CaCl2, 2.5; EGTA, 0.06; NaATP, 1; HEPES, 2; sucrose, 20; adjusted to a pH 7.4 with KOH. The pharmacological agents were applied by pressure ejection from a second pipette positioned near the cell. We studied 41 bipolar cells of which 14 showed no response to L-glutamate (1-10 µM), and the rest of the cells fell into two classes: 9/23 of the papain dissociated cells responded with a large inward current that reversed around +10 mV; in addition, 3/3 of papain-dissociated cells showed inward currents in response to ketamine (25-50 µM). In contrast, 4/23 of the papain- and 13/19 of the dispase/collagenase-dissociated cells responded with a large inward current that reversed around +10 mV. 4/4 of the dispase/collagenase-dissociated cells tested showed outward currents to APB (2-amino-4-phosphono butyrate, 50 µM), a known agonist for ON-bipolar cells (Slaughter & Miller, J. Neurosci., 5:224, 1985). Multipolar cells did not show this difference depending on dissociation procedures; all of the responding cells (27/35) gave a clear inward current in response to glutamate (1-10 mM). The glutamate-evoked currents of opposite polarity we observed in the bipolar cells may underlie the physiological basis of the ON- and OFF- responses in these second-order neurons. [Supported by the Lucille P. Markey Charitable Trust, NIH EY05201, the Klingenstein Fund, and an Avitar Center Award (NS-23785).]
PERMEABILITY OF GLUTAMATE-GATED CHANNELS IN RETINAL BIPOLAR CELLS.

A. L. Dong, T. J. McBurney, Dept. of Biophysics, SUNY, Buffalo, NY 14214.

Recording intracellularly in the superfused tiger salamander retina, we have found that baclofen, a GABA-B receptor agonist, makes amacrine and ganglion cells respond more transiently to a step of light. In third order neurons that normally respond transiently, baclofen enhances responses to diffuse illumination. The cells that normally respond in a sustained manner to a step of light, baclofen application often suppresses the sustained responses and the cells begin to respond like transient neurons. Since tonic and phasic responses can carry very different information, the action of this GABA analog suggests that response characteristics of retinal neurons could be under synaptic control. To test this, we looked at trigger features that might be induced by baclofen. We found that baclofen modified the response properties of some cells that normally respond transiently to a moving slit under control conditions, a pronounced directional selectivity became evident after baclofen treatment. This change in response properties may be a form of selective attention. If baclofen’s action is related to selective attention, then it should under effective control. Dr. S. Ball has anatomical evidence of efferent inputs to GABAergic amacrine cells, which could potentially serve this function (pers.com.). NEI EY05725

CORTICOTROPIN-RELEASING FACTOR-INDUCED CURRENTS IN ACUTELY DISOCIATED RAT RETINAL GANGLION AND BIPOLAR CELLS.

Hermes H. Yeh, Department of Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642.

In the rat retina, corticotropin releasing factor (CRF)-like immunoreactivity has been localized to a subpopulation of amacrine and displaced amacrine cells. In this preliminary study, whole-cell patch clamping was used to determine whether CRF could elicit current responses in isolated ganglion and bipolar cells.

Bipolar cells were identified by the presence of rhodamine-labeled microspheres injected into the superior colliculi 2-4 days prior to the experiment. In the physiological potential range, application of the high dose (50-100 µM) CRF evoked inward currents of 3-5% of the control input resistance in 2 of 21 bipolar cells. We have not yet determined the pharmacological characteristics of these CRF-induced inward currents.

Corticotropic factor (CRF) is a newly described hormone that is involved in the regulation of the hypothalamic-pituitary-adrenal axis. It is believed to be involved in the central nervous system, and may serve as a neurotransmitter.

In our study, we used corticotropin-releasing factor (CRF) to test its effect on retinal ganglion and bipolar cells. We observed CRF-induced inward currents in a subset of cells, indicating that CRF may act as a neurotransmitter in the retina. Further studies are needed to confirm these findings and to determine the physiological role of CRF in the retina.
396.9
EFFECTS OF MUSCIMOL ON CAT RETINAL GANGLION CELL ACTIVITY. W. P. Przybylszefski, M. Hagner* and H. Sacher*. Dept. of Physiology, Free University, Berlin, Arndtallee 22, 1 Berlin 33, Germany. [SPON: European Brain and Behavior Society].

396.10
RESPONSES OF CAT RETINAL GANGLION CELLS TO EYEBALL DEFORMATION DURING DIFFERENT STAGES OF DARK ADAPTATION. M. Hagner*, A. W. Przybylszefski and O.-J. Grüter. Dept. of Physiology, Free University, Arndtallee 22, 1000 Berlin 33, Germany. [SPON: European Brain and Behavior Society].

396.11

396.12

396.13

396.14
A NEURAL NETWORK MODEL OF VISUAL RECEPTIVE FIELD REGIONS AS A FUNCTION OF RESPONSE LATENCIES. L. Sun* and E. Michail-Ramakou. Dept. of Biomedical Engineering, Rutgers University, P.O.Box 909, Piscataway, New Jersey 08855-0909.
396.15 INTERACTION BETWEEN NEUROPEPTIDES AND GLYCINE IN THE AVIAN RETINA. T. Li*, D. M. K. Lam and Y. Y. T. Su. Center for Biotechnology, Baylor College of Medicine, Houston, TX 77030.

Previous studies from this laboratory have demonstrated the interaction between enkephalins and GABA, glycine or dopamine in the chicken retina (Su et al., Biochemistry, 35:2305, 1996). We present our recent studies on the interaction between somatostatin, glycine, neurotensin and glycine in the chicken retina. Our preliminary results showed that in the presence of 2 µM of somatostatin approximately 32 ± 43% of K+-induced 3H-glycine release was inhibited, while the same dosage of neurotensin increased 3H-glycine release by 36 ± 22%. This suggested these peptides and glycine are functionally related. The structural basis of these systems was studied using double-label techniques. In addition to those cells which were labelled for each marker alone, some of the cells were found to be double-labelled for both 3H-glycine and somatostatin or neurotensin-immunoreactivity. The neurotransmitter and glycine-stained processes, which may have originated from single- or double-labelled cells, overlapped extensively in the inner plexiform layer. The interaction between these systems may be confined to this layer, where somatostatin may function as a presynaptic inhibitor and neurotensin as a presynaptic activator of glycine accumulating terminals.

396.17 GLYCINE STIMULATES CALCIUM INDEPENDENT RELEASE OF 3H-GABA FROM Xenopus laevis retina. John F. Smiley* and Scott F. Basinger, Program in Neuroscience and Cullen Eye Institute Baylor College of Medicine, Houston, TX 77030.

We have previously characterized an interplexiform cell in the Xenopus laevis retina that is labeled by both glycine high affinity uptake and somatostatin antibodies. We are now using the function of these two putative neurotransmitters by monitoring the release of retinal 3H-GABA, presumably from horizontal cells. Retinas are incubated 10 minutes in a bath containing 10 nM 3H-GABA, perfused for 32 minutes, and then drugs are added to the perfusate. In most experiments six consecutive 4 minute pulses of drug are applied, with 10 minutes wash between pulses. Micromolar concentrations of glycine or glutamate are seen to stimulate 3H-GABA release with approximately equal potency. These effects are completely independent of Ca2+, as seen in Ringers with 0Ca2+/2mM Mg2+. One difference between these responses is that glutamate down-regulates itself, causing substantially smaller responses in the repetitive pulses. In contrast the glycine response at first increases, and then only gradually decreases. These patterns of self-regulation are unaffected by Ca2+ concentration. The response to glycine is inhibited by strychnine. Potassium also stimulates release, but this is about 75 Ca2+ dependent. Somatostatin-14 or -28 have no effect on concentrations of 10mM to 2uM. (Supported by NIH grants.)

396.18 GLYCINE INDUCED REVERSIBLE BLINDNESS IN SHEEP. N. Wright, R. Hill, J. Seggie (IPAF: M. Stainier). Departments of Biomedical Sciences, Psychiatry, Medicine and Pathology, McMaster University, Hamilton, Ontario L8N 3S5.

The use of a 1.5% glycine solution as a bladder irrigant during surgical removal of the prostate has been associated with transient visual impairment. Glycine is thought to be an inhibitory retinal neurotransmitter. Adult female sheep (N=17) were infused with 0, 500, 1000, 2000 or 4000 ml of 1.5% glycine. The volume control was a solution of dextrose and saline. The pupil response to 5 seconds of bright light following dark dilation was used as an index of retinal response. Observations were made at 2, 4, 6, 12, 24, 48 and 96 hours after infusion. Glycine infusion resulting in plasma levels up to 15,000 µmol/L inhibited pupil response to light is a dose dependent manner. Inhibition of pupil response paralleled by behavioural indices of visual impairment and blindness but not by changes in plasma sodium, chloride, potassium or osmolality. The duration of effect was also dose dependent with maximal impairment following 4000 ml of glycine being detectable up to 24 hours. Data suggest that glycine inhibits retinal responsiveness to light in a dose dependent and reversible manner.

Supported by the St. Joseph's Hospital Foundation, Physicians Services Inc. and Baxter Inc. JS is an Ontario Mental Health Foundation Research Associate.

396.19 GLYCINE AND GABA ACTION ON THE SCOTOTIC THRESHOLD RESPONSE OF THE RABBIT SCOTOTIC ERG. F. Olivier, P. B. Jolicoeur, G. Lafond*. A. Drumheller and J. Bruneau*. Departments of Ophthalmology and Psychology, Faculty of Medicine, Sherbrooke University, Sherbrooke, Quebec, Canada J1H 5N4.

In order to better understand dopaminergic functions in mammalian retina we initiated a series of experiments in which the effects of several doses of the well known dopamine agonist apomorphine (A) were tested in the rabbit scotopic ERG. A was administered intravenously in different groups (N=10) were studied in dark adapted rabbits and compared to a control group of animals (N=10) that received the same volume of the vehicle solution. ERG parameters investigated included amplitudes and implicit times of A- and B-waves as well as the oscillatory potentials A, B1 and B2. The smallest dose (0.01 mg) significantly increased amplitudes at all intensities studied. The intermediate dose (0.1 mg) was without any effects whereas the largest dose (1.0 mg) markedly decreased amplitudes. A was potent at high intensities of stimulation. It is noteworthy that on oscillatory potentials, apomorphine produced an enhancement of OP2 and OP3 amplitudes only with the intermediate dose (0.1 mg). Other oscillatory potentials were unaffected by the drug.

Together, our results constitute experimental evidence that retinal dopaminergic neurons implicated in the generation of the B-wave and specific oscillatory potentials amplitudes possess presynaptic inhibitory receptors which are stimulated by relatively small doses of a dopamine agonist. The fact that A-waves, CP1 and CP4 amplitudes or implicit times of all ERG parameters remained unaffected suggest that retinal dopamine autoreceptors are differentially implicated in retinal response to photic stimulation. Supported by MRC grants MT 2593 and DG 264.
397.1

THE VISUAL WULST MODULATES THE DIRECTIONAL SELECTIVITY OF ACCESSORY OPTIC UNITS IN THE PIGEON. Heiji El-Hamamsy* and O.C. Gasparotto*. Dept. of Physiology, Federal University of São Paulo, São Paulo, Brazil.

The visual telencephalon is known to project to accessory optic system (AOS) nuclei. In order to verify the impact of the AOS function, we examined the directional selectivity of units within the nucleus of the basal optic root (nBOR) of the pigeon AOS, before and after visual Wulst lesions. Thirty adult pigeons (Columba livia) were urethane-anesthetized and subjected to monocular dynamic photic stimulation. Half such birds previously suffered unilateral or bilateral Wulst ablations. Directional selectivity of nBOR units recorded with tungsten electrodes was then evaluated in terms of a vector analysis. In the normal pigeon, most nBOR neurons displayed preferences to upward-temporal or downward-nasal motion. After ipsilateral or bilateral Wulst lesions, the upward response components were almost absent, with most units now preferring temporal or downward-nasal motion. The contralateral lesions generated, instead, a depression for random dots stimuli presented to the contralateral eye in a temporal direction for random dots stimuli presented to the contralateral eye. Many units were excited by stimulus presentation in both the preferred and null directions. The direction selectivity of the cells was very pronounced for a wide range of stimuli. The ratio of preferred to null responses ranged from 4:1 to 25:1. Supported by CNPq, FAPESP and FINEP grants.

397.2

FOURIER ANALYSIS APPLIED TO VISUAL NEURONS TO DETERMINE DIRECTIONALITY. B. SIMINOFF, UNIVERSITY OF MASSACHUSETTS AMHERST, MA (SPONSOR J. Desmond).

A neuron displays directional symmetry (DS) if (a) there is a preferred direction (PD) to stimulus moving across the receptive field, (b) there is a null direction (ND) 180 degrees out of phase, and (c) responses decrease monotonically and symmetrically from the PD to ND. Degrees of directionality of 110 neurons located in the pretectum of the frog (Pile et al., 1987) using gratings moving at 6, 15 and 25 deg/sec and 8 orientations are evaluated using Fourier Transforms. Practically all units that do not decrease from the PD to ND as a straight line function. PD's are independent of velocity in 75% of the neurons, and are along the nasal-temporal and vertical axes in 32 and 15% of the neurons, respectively. ND's are 180 and 90 degrees out of phase in 30 and 40% of the neurons, respectively. While directionality is independent of background activity, a unit that is "broadly-tuned" can become "narrowly-tuned" if background activity is increased since the background activity sets the zero level.

397.3


Single-unit recordings were performed on eight adult pigeons subjected to a midline pretrigeminal section. The electrodes were oriented to the brachium of the superior colliculus, the optic tract and the posterior commissure. Male pigeons (C. livia) were anaesthetized with halothane and electrocuted and then perfused with 4% paraformaldehyde. The brain was embedded in 70% glycerol and cryosectioned at 50 microns. Immunostaining for the neurotransmitter dopamine was performed with antibodies to the dopamine receptor D2 and to the dopamine transporter. The results show that virtually all MTN neurons in the posterior comissure are projection neurons. Supported by NSF grant #MA 7244 to B.J.F.

397.4


Previous research has shown that the nBOR is involved in the processing of visual information for compensatory head and eye movements. It has also been reported that these neurons have large receptive fields and respond best to large visual stimuli moving slowly in a particular direction. Two classes have been described, those preferring movement up and those preferring movement down along the vertical axis. The present study confirms these findings, but describes several classes of neurons. Broadly tuned neurons have high spontaneous rates, but many narrowly tuned neurons have low spontaneous rates. Subfield stimulus presentation indicates that responses are not uniform across the receptive field, that is, there is an area of maximum excitation. Furthermore, the minimal area for a directional response and the area for a maximum response vary considerably. This research was supported by NRC grant #MA 7244 to B.J.F.

397.5

CELLS IN THE TURTLE'S BASAL OPTIC NUCLEUS ARE DIRECTION SELECTIVE OVER A BROAD VELOCITY RANGE. A.F. Rosenhabs and M. Arie, Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Retinal direction selective (DS) ganglion cells are thought to provide input to a neural circuit controlling optokinetic nystagmus through the BON of the accessory optic system. Since the BON is a small ventral brainstem structure lying directly above the cranial floor, in vivo recordings are difficult. In our experiments, we use a novel in vitro brain preparation on a previous description of retinal-olivary projecting cells. Most of the units showed excitation for random dots stimuli presented to the contralateral eye in a temporal direction and inhibition in the opposite direction. An average of 45% of the units responded to the ipsilateral eye, with two exceptions, which had the strongest excitatory response at opposite directions for each eye. Both eyes showed the same overall pattern of directionality. Inhibition was stronger than excitation in most ipsilaterally responding cells. Excitatory response to the ipsilateral eye was always weaker than that elicited by the contralateral eye in a few cases the ipsilateral inhibitory response was stronger. Many units were excited by stimulus velocities ranging from 0.6 to 150 deg/s. Inhibited responses were turned to intermediate speeds. The response properties of these units show similarities and discrepancies with other species that can be correlated with the differences in the performance of the optokinetic reflex.

397.6


Most neurons of rat medial terminal nucleus (MTN) project to the nucleus of the optic tract (NOT), but some project to other brainstem nuclei involved in controlling eye movements. The possibility that MTN-NOT neurons collateralize to innervate other MTN targets is studied with two retrograde fluorescent tracers. Fluorogold was injected into the NOT, and Fast blue was injected into one of the following nuclei: the ipsilateral supraoculomotor nucleus, the ipsilateral aqueductus mesencephali, and the contralateral oculomotor nucleus. The retrograde and anterograde labelling is studied with two retrograde fluorescent tracers. The results show that nearly all MTN neurons are single-labelled in all paired injections of the NOT and each of these other nuclei. About 10% of MTN cells project to the NOT and 4-9% to each of the other nuclei. Only around 1% of MTN cells are double-labelled showing that the MTN- NOT neuron population is distinct from neuron pools projecting to the other outflow areas. These data reveal that virtually all MTN neurons are projection neurons. Supported by NSF grant BNS-8612919 and NIH grant NS-13521. KJC is a Brazilian National Research Council (CNPq) Scholar.
397.7

The supragateculepretal nucleus (SgP) in the rabbit consists of large darkly stained cells located posterior to the nucleus of the optic tract (NOT). Injection of 3H-leucine into the SgP of rabbits shows that it projects ipsilaterally to: stratum griseum profundum (SGP) of SC, stratum griseum and profundi (SGP) of SC, olivary pretectal (PO), NOT, medial pretectal area, periacuicular gray, thalamic supragateculeate, and lateral hypothalamus. The anterior pretectal nucleus was conspicuous by the absence of either fiber or terminal label. Contralateral projections are to SgC, PO, SGP and SgP. Injection of HRP into the SgP shows the SgP-SgC bilateral connections of the SgP and also shows that the SgP projects to the ipsilateral SgP. The only cortical label was in the temporal area and was probably due to leucine uptake in the medial geniculate. Thus, the rabbit SgP is a heterogeneous nucleus with which in the cat has projections to the insular cortex. Supported by NSF Grant 88-162319 to RAG & BH8.

397.9
GABA-ERGIC INPUTS TO THE NUCLEUS ROTUNDUS IN PIGEONS (Columba livia). T. Skeen, T. J. Karten, and W. Windheisser. Department of Neurosciences, M-908, School of Medicine, University of California, San Diego, La Jolla, California 92093.

The nucleus rotundus thalami (Rt) in birds plays an important role in visual information processing. Rt receives a non-topographic bilateral projection from layer 13 of the optic tectum, as well as from the subpretectal complex (SP/IPS) and retinorecipient superior thalamic nucleus (Rt). Rt projects to the ectorhinostriatum, a visual structure in the avian telencephalon (Benowitz and Karten, J. Comp. Neuro., 167:93, 1976).

Antibodies directed against glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of gamma-aminobutyric acid (GABA), labeled a major axonal plexus in Rt, but did not label any somata. Immunohistochemical and neuroanatomical tracing techniques were used to determine the somata of this inhibitory neurotransmitter. HRP and WGA-HRP immunoactivity was not found in somata of layer 13 of the tectum, but was present in somata of SP/IPS and Rt. When the anterograde tracer phasodase vulgaris leucoagglutinin (PHA-L) was injected into layer 13 of the tectum, dendria PHA-L positive axons were found in Rt. These axons, however, did not express GAD immunoactivity. When PHA-L was injected into SP/IPS, we found that some PHA-L positive axons in Rt were also GAD positive. Lesions of SP/IPS caused a significant decrease of GAD positive axons in Rt.

Thus, the source of GAD positive axons in Rt is at least partially from SP/IPS, not the optic tectum. Since SP/IPS itself is a target of the test efferents from layer 13, the SP/IPS-retinal pathway may provide feed-forward inhibitory information to the testo-rotundo-ectostriatum system. Supported by NINCDS NS25600 to HJK.

397.11
RETINOTOPIC ORGANIZATION OF OPTIC PROJECTIONS IN MONKEY VISUAL CORTEX, R. Fite and N. Montgomery, Neurosciences & Behavior Program, University of Massachusetts/Amherst.

The retinotopic organization of the anuran visual system has been reinvestigated using anterograde and retrograde transport of HRP following small retinal lesions, as well as restricted HRP injection of tectum and pretectum. The Ferret visual claustrum are sensitive to both of stimulus form. These findings support the hypothesis of stimulus form. These findings support the hypothesis of Claustral neurons respond to a broad range of speeds which show that retinotopic mapping is maintained over the range of speeds where directional sensitivity is manifest. Further, the remaining neurons that were not directional selective were sensitive to a range of speeds and were more likely to be sensitive to a range of speeds which were only slightly different in direction. These findings support the hypothesis of monocular neurons which show directional sensitivity to a range of speeds. The remaining neurons that were directionally selective when tested at the preferred speed did not show any directional preferences when tested at other speeds. Claustral neurons respond to a broad range of speeds and appear suited for the detection of motion and not for the detection of preferred directionality across a broad range of speeds may be involved in the detection of the direction of motion. These findings support the hypothesis of Boyapati and Henry (1985) that the claustrum is involved in motion detection. Supported by NHEC grant to BJF (A0353).

397.12

The lentiform nucleus of the mesencephalon (LM) is a primary structure that receives inputs from the retina, which we have shown to be spatially organized (JCN 26/3 98) and from the tectum. In this study we used the HRP retrograde tracing technique to determine the topographic projection arising from the optic tectum to the LM in chickens is also spatially organized and to demonstrate the cells of origin. A single population of labeled neurons, with soma size measuring 67.9 ± 2.2 um in average, was identified after injections into the LM. The projection neurons were confined to the lateralmost portion of the ipsilateral accessory hyperstriatum (UH) throughout its caudal to rostral extent, except for the rostral portion of LM. Partial injections into the LM revealed a spatial organization of the projection neurons. Labelled neurons in the caudal two-thirds and in more ventral portions of the LM projection area were project to the doral LM. Labelled neurons throughout the entire rostral-caudal extent of the cell projection area, but in more dorsal region caudally, projected to the ventral LM. These findings suggest that a single population of projection neurons, which is confined to a specific portion of the LM, project in a spatially organized manner to the LM. Thus study, in conjunction with electrophysiological evidence for a retinotopic organization of the LM has lead to the possibility that the LM receives a spatially organized projection from the retina, either indirectly through the HA of the visual thalamus. The LM in mammals, which is considered homologous to the LM, also shows a spatial organization of its afferents arising from the visual cortex. Supported by NIH EY 03613. 
SUBCORTICAL VISUAL PATHWAYS IV
THURSDAY AM

397.13

The centrifugal fibers projecting to the retina originate in the isthmo-optic nucleus (ION). ION neural activity is strongly driven by a retinotopically organized visual input from the ipsilateral tectum, and, if found, is also modulated by saccadic eye movements. Since degeneration studies years ago suggested an input to the ION from the optic motor nucleus, we reexamined the afferent connections to the ION using the HRP technique.

The ION of four-week-old chickens was injected using KCl-HRP-filled micropipettes, after localizing the nuclei by recording their visually evoked neuronal activity. Injects were small and restricted to the ION.

We found no trace of retrogradely transported HRP in any of the optic motor nuclei. Only cells in the ipsilateral tectal complex were consistently labeled. The majority of the neurons had cell bodies located in lamina h of the stratum gisum et fibrum superficialis. The large axis of the dendritic arborizations was perpendicularly to the tectal laminae, with shorter dendrites extending upward, toward lamina g, and thicker and longer ramifying branches extending downward. Due to no dendrites were observed above lamina g, where retinal fibers terminate, tecto-ION cells in the chicken do not receive a direct projection from the retina. Instead, their larger dendrites directed toward a deeper layer suggests that the visual input to the tectum is integrated with other inputs, including from the saccade-generating circuitry, to form the output to the ION. Experiments addressing this possibility will be presented.

397.15

During a study of fiber order in the ferret retinotopical pathway we have noticed that the diameters of the largest axons in the optic tract are greater than those in the intrabulbar optic nerve. Since we have quantified this and shown now that in the retina there are axons whose diameters greater than that of the largest found in the post-optic segment of the nerve. Vibrations sections (200µm) from the intrabulbar optic nerve and optic tract were cut and the diameters of these axons were measured by the Bioquant image analysis system.

Counting the fiber with inside diameter greater than 3.2 µm (D = perimeter/π) gave the following results: Ferret 1: intrabulbar optic nerve = 304 fibres, tract = 462 fibres. Ferret 2: intrabulbar optic nerve = 83 fibres, tract = 462 fibres. These large fibers in the tract must be retinofugal since they are larger than the average retinal fiber order in the tract and are likely to be the fibers that form the major pathway from the retina to the superior colliculus. The thickness of these fibers is likely to be greater than that of the fibers that form the major pathway from the retina to the superior colliculus. The thickness of these fibers is likely to be greater than that of the fibers that form the major pathway from the retina to the superior colliculus. The thickness of these fibers is likely to be greater than that of the fibers that form the major pathway from the retina to the superior colliculus.

397.16
THE RELATIONSHIP BETWEEN THE PROJECTIONS FROM VISUAL AREAS VI AND VI TO THE THALAMIC RETICULAR NUCLEUS IN THE RABBIT. H. W. Crabtree. (SPH: H. W. Crabtree, Dept. of Psychology, Mich. State Univ., East Lansing, MI. The traditional view of the thalamic reticular nucleus (TRN) is that adjacent areas of the overlying neocortex map onto adjacent areas within the plane of the TRN. A previous study (Crabtree, H. W. and Killackey, H. F., Neurosci. Suppl., 21: 804, 1987) showed that, in the visual sector of the rabbit's TRN, focal areas of visuo-cortical area VI are represented by "slabs" of TRN underlying the visual sector of the nucleus in the rostrocaudal dimension. In the present study of the rabbit, projections of HRP in the TRN were studied and compared with those following tracer injections into VI. A single injection in VI results in a single focus of label that is located in the dorsocaudal visual sector of the nucleus. The projected label is restricted to VI produce slabs of label that are confined to the outer two-thirds of the sector, while those involving VI result in slabs of label that are confined to the outer two-thirds of the sector. Thus, the neocortex is represented in a discontinuous fashion along the plane of the TRN. The target of the afferents from the inner aspect of the TRN's visual sector, which receives the optic tracts, remains to be defined. (Supported by the WCR, Grant No. S34037)

397.17
ULTRASTRUCTURAL EXAMINATION OF TECTAL TERMINATIONS IN THE VENTRAL LATERAL GENICULATE NUCLEUS (vLGn) IN THE CAT. J. D. Rosemullow & G. D. Patlaj, Department of Biomedical Sciences, University of Guelph, Guelph, Ont., N1G 2W1.

In further understand the visual process, the functional morphology of the ventral lateral geniculate nucleus (vLGn) was investigated. Tectal input was examined using both retrograde and anterograde tract labeling techniques. The anterior pole of the superior colliculus was injected with HRP in 7 cats with care to avoid damage to the overlying visual cortex. These injections were performed 5-6 weeks after the surgery. The injection site was carefully avoided to minimize the damage to the superior colliculus. The injection site was carefully avoided to minimize the damage to the superior colliculus. The injection site was carefully avoided to minimize the damage to the superior colliculus. The injection site was carefully avoided to minimize the damage to the superior colliculus.

Degenerating boutons were localized in this region. Qualitatively, they were mostly axo-axodendritic, type F connections to the LGn. This type is characterized by thicken flattened vesicles, which are characteristic of an inhibitory synapse. Preliminary results indicate that normal synaptic organization is maintained, although most frequently of this classification. This convey the possibility of an inhibitory role by the tectal afferents to the LGn, with combined visuomotor-vestibular function, such inhibition is yet to be functionally quantified.

397.18

The ontogenetic and comparative cytoarchitectonic studies of Rose ('42) led him to conclude that the ventral thalamus consists of the ventral lateral geniculate nucleus (vLGn) and the thalamic reticular nucleus (TRN), and that reticular portions of GLV were often confused with the more rostral regions of the thalamus. In this study, we have used retrograde and anterograde tract labeling techniques to study the functional organization of the vLGn in the tree shrew and have identified 5 subdivisions of the vLGn, each with specific connections, supporting Rose's view: the external segment and intergeniculate leaflet are retinofugal- and tecto-recipient and contain numerous, interneuronal for synaptic pairs, and regions with sparse terminal fields; the internal segment projects to the tectum and anterior pretectal nucleus and, with the external segment, receive projections from the cerebellum and projects to the nucleus reticularis tegmenti pontis. Supported by NIH MHO 59985, and MH20060 NSB 407779 and "Deutsche Forschungsgemeinschaft".
THE VENTRAL LATERAL GENICULATE IN A LIZARD.
Neil W. Montgomery & Robin Mergendahl*
University of Massachusetts, Amherst, Mass.01003

The term ventral lateral geniculate (VGL) is used extensively to describe a visual nucleus in the ventral thalamus of a number of vertebrates yet the relationship between these structures is unknown. In the present study the connectivity of VGL in Anguis carolinensis has been investigated using HRP.

In lizards the VGL is a large crescent shaped structure consisting of a medial cell plate and lateral neuropil. The neurons of the cell plate have paleissalike dendritic fields while the neuropil contains stellate neurons. The neuropil receives retinal afferents while the cell plate receives further, the telencephalic map in VGL is topographically organized and is a 'mirror-image' of that found in the tectal lobe.

Two features from the ventral thalamus of Anguis: a) the excitability of remaining hippocampus, and b) a 'mirror-image' of that found in the tectal lobe.

The author was an MRC Scholar and is presently an MRC Scientist.

CEREBRAL ISCHEMIA IV


The CA1 and CA3 subfields of hippocampus are often necrotic following disease processes, particularly temporal lobe epilepsy. We have been interested in the effects such selective cell loss has on the physiology and restructuring of remaining hippocampal neurons and in the relevance these processes may have for the clinical population with similar damage.

Selective loss of CA1 is produced experimentally, and has been observed in humans, following transient ischemia. We used this approach, produced in adult male Wistar rats by 15 minutes of carotid artery occlusion with vertebral artery cautery, to examine the consequences of CA1 loss on remaining dentate and hippocampal neurons. We have observed that ischemia results in near-complete loss of the CA1 neurons. The remaining hippocampus is now irregularly topographical throughout the hippocampus and dentate gyrus. This loss was observed up to 6 weeks following ischemia and appeared to be specific in that CA1 immunoreactive neurons were still apparent. Studies are currently ongoing to determine if reduced somatostatin represents selective cell death or decreased peptide expression.

Somatostatin has been proposed to play a critical role in modulating inhibitory interneuron function. Selective loss of this population of cells or of the peptide they contain, particularly in regions where principal cells remain intact, may alter the excitability of remaining hippocampus.

Supported by NHR-NINDS grants NS20482 and NS25155.

ROLE OF LACTIC ACID TRANSPORT IN THE CNS. W. Neill. Dept. of Physiology, Coll. of Med., Univ. of Saskatchewan, Saskatoon, S7N OWO, Canada.

Lactate/lactic acid is thought to be a mediator of reversible brain damage during anoxia and ischemia. Lactate release of cultured astrocytes and of cultured neurons was investigated. The internal lactate concentration remained stable at 25 mM in both cell types. A lactate/proton cotransport mechanism was found to operate in both cell types. They therefore will excrete lactic acid into the ECS. The release rate, but only after swelling was accomplished.

Content did not change, confirming that lactate is not involved in brain cell swelling. The action of lactate/lactic acid on neuronal transmission in the hippocampus was investigated. Lactate (30 mM) did not lead to lactic acid, however, lactic acid to a decrease in pH, damaged synaptic transmission irreversibly. Thus, lactate as an anion can be very well tolerated by the CNS. However, lactic acid will damage synaptic transmission.

The author was an MRC Scholar and is presently an MRC Scientist.

ELECTRICAL, ION TRANSPORT AND METABOLIC CHANGES DURING BRAIN ISCHEMIA IN RAT: COMPENSATORY BUT NOT THRESHOLD EVENTS. C.N. Raffin*. M. Harrison*. T.J. Sick and M. Rosenthal. Dept of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Ligation of the carotid arteries after electrocoagulation of the vertebral artery (the 4-vessel occlusion model of Pulsawski and Breitlower) produces an event sequence in rat brain that includes EEG suppression, anoxic depolarization (AD) with maximal increases in extracellular potassium ion activity (K+) and maximal decrease in tissue oxygenation (tPO₂) with reduction of the mitochondrial electron transport carriers. To determine whether these changes are predictive or determinate of subsequent events, and to provide insights into mechanisms of EEG suppression and AD, electrode and optical techniques were employed in rats anesthetized with pentobarbital. No threshold levels of cytochrome c α₃ reduction, tPO₂ or K⁺ could be determined for EEG suppression. Also, no threshold values of tPO₂, reduction of cytochrome c α₃ or K⁺ could be found that were predictive of AD onset. However, latency to EEG suppression was inversely correlated with latency to AD, maximal decreases in tPO₂ and maximal reduction of cytochrome c α₃. In contrast, latency to AD was proportional to latency of subsequent maximal decreases in tPO₂ and cytochrome c α₃ reduction. These data do not support the concept that EEG suppression and AD are each produced by thresholds derangements of mitochondrial function. On the contrary, they suggest that EEG suppression is a compensatory process in space oxygen for the production of energy for other activities such as ion transport and to avoid the consequences of the loss of ion homeostasis. The early suppression of EEG also suggests the activity of a sensor of potential energy failure, the identity of which, in ischemia, remains unknown.
CEREBRAL ISCHEMIA IV  THURSDAY AM

TRANSPORT INHIBITORS, ON THE RELEASE OF PURINES FROM RAT NEURONAL ACTIVITY, INDUCE SLEEP IN DOGS, DEPRESS LOCOMOTOR ENHANCING ADENOSINEREGIC TONE HAS BEEN POSSIBLE USING EVOKED ADENOSINE, INOSINE, HYPOXANTHINE AND XANTHINE ACTIVITY IN MICE, EXERT ANTICONVULSANT EFFECTS AND ENHANCE SYNTHESIS AFTER 5 MIN. ISCHEMIA. HIPPOCAMPAL NE FELL FROM 0.29 ± .04 AT 1 HR. IN SHAM OPERATED CONTROLS. AT 24 AND 48 HRS. POST-OPERATION, NE LEVELS HAD RETURNED TO BASELINE. TO DETERMINE WHY NE WAS DECREASED AFTER ISCHEMIA, THE DOPA DECARBOXYLASE INHIBITOR NSD-1015 WAS USED TO ESTIMATE NE SYNTHESIS. DOPA IS BARELY DETECTABLE IN THE NORMAL BRAIN, BUT 30 MIN. AFTER NSD-1015 ADMINISTRATION 13 ± 10% ACCUMULATES. NEVUS OR DOPA IS A good CANDIDATE AS A NE SYNTHESIS INHIBITOR. ASSAYING FOR NEUS OR DOPA IN THE DIALYSATE FROM HIPPOCAMPAL SUBFIELD CA1 AND PERIFACIAL CSF, ISCHEMIA WAS INDUCED IN 5 RATS BY HYPOTENSION AND CAROTID CLAMPING (2-V-0 MODEL) FOR 30 MIN. TEN MINUTE FRACTIONS OF DIALYSATE WERE COLLECTED POST-ISCHEMIA AND DIAPOSED TO A HIGH-SENSITIVITY RADIOIMMUNOASSAY (RIA). THE SPONTANEOUS RELEASE OF NAAG-LIKE IMMUNOREACTIVITY (NAAG-LIR) WAS HIGH (16.8 ± 2.3 PPM) IMMEDIATELY AFTER PLACEMENT OF THE PROBE. NEUROTRANSMITTER RELEASE WAS MEASURED IN VARIOUS REGIONS OF THE BRAIN. THE RESULTS INDICATE THAT THE RIA METHOD IS SUFFICIENTLY SENSITIVE TO MEASURE ENDOGENOUS NAAG IN DIALYSATE. THE NAAG RELEASE DURING ISCHEMIA SUGGESTS THAT NAAG MAY PLAY A ROLE IN MEDIATING ISCHEMIC NEURONAL INJURY.

DECREASED HIPPOCAMPAL NOREPHINEPHRINE AND IMPAIRED SYNTHESIS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE MOUSE. J.R. Insel*, F.E. Moore and J.N. Davis. Institute of Medicine (Neurology) and Pharmacology, V.A. and Duke Univ. Medical Centers, Durham, NC. Hipocampal, neocortical, and caudate neurons are selectively vulnerable to transient ischemic injury. We measured the level and rate of NE synthesis after 5 min. ischemia. Hippocampal NE fell from 0.29 ± 0.14 nf/mg 1 hr. following ischemia, compared to 0.21 ± 0.12 1 hr. in sham operated controls. At 24 and 48 hrs. post-surgery, NE levels had returned to baseline. To determine why NE was decreased after ischemia, the dopa decarboxylase inhibitor NSD-1015 was used to estimate NE synthesis. DOPA is barely detectable in the normal brain, but 30 min. after NSD-1015 administration 13 ± 10% accumulates. NEVUS OR DOPA IS A GOOD CANDIDATE AS A NE SYNTHESIS INHIBITOR. ASSAYING FOR NEVUS OR DOPA IN THE DIALYSATE FROM HIPPOCAMPAL SUBFIELD CA1 AND PERIFACIAL CSF, ISCHEMIA WAS INDUCED IN 5 RATS BY HYPOTENSION AND CAROTID CLAMPING (2-V-0 MODEL) FOR 30 MIN. TEN MINUTE FRACTIONS OF DIALYSATE WERE COLLECTED POST-ISCHEMIA AND DIAPOSED TO A HIGH-SENSITIVITY RADIOIMMUNOASSAY (RIA). THE SPONTANEOUS RELEASE OF NAAG-LIKE IMMUNOREACTIVITY (NAAG-LIR) WAS HIGH (16.8 ± 2.3 PPM) IMMEDIATELY AFTER PLACEMENT OF THE PROBE. NEUROTRANSMITTER RELEASE WAS MEASURED IN VARIOUS REGIONS OF THE BRAIN. THE RESULTS INDICATE THAT THE RIA METHOD IS SUFFICIENTLY SENSITIVE TO MEASURE ENDOGENOUS NAAG IN DIALYSATE. THE NAAG RELEASE DURING ISCHEMIA SUGGESTS THAT NAAG MAY PLAY A ROLE IN MEDIATING ISCHEMIC NEURONAL INJURY.

EVIDENCE FOR IN VIVO RELEASE OF N-ACETYLGLUTAMATE FROM THE RAT HIPPOCAMPUS DURING CEREBRAL ISCHEMIA. J.K. Deshpande, G. Tsai, R.J. Trayanov* and J.T. Coyle. Depts. of Anesthesiology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cerebral ischemia causes excitotoxicity in select brain regions such as the hippocampus, possibly through the activation of glutamatergic receptors. N-acetylaspartylglutamate (NAAG) is a neuropeptide localized to some glutamatergic pathways and may act as a neurotransmitter/modulator of excitatory glutamate NMDA receptors. To determine whether NAAG is released during transient cerebral ischemia, microdialysis probes were stereotaxically placed into hippocampal subfield CA1 and perifacial CSF. Ischemia was induced in 5 rats by hypotension and carotid clamping (2-v-0 model) for 30 min. Ten minute fractions of dialysate were collected post-ischemia and analyzed for NEVUS and DOPAC. NEVUS was decreased after ischemia, the DOPA decarboxylase inhibitor NSD-1015 (10M) was perfused through the probe and its effects on neurotransmitter release were measured. NEVUS release was decreased, the DOPA decarboxylase inhibitor NSD-1015 (10M) was perfused through the probe and its effects on neurotransmitter release were measured. NEVUS release was decreased and the DOPA decarboxylase inhibitor NSD-1015 (10M) was perfused through the probe and its effects on neurotransmitter release were measured.
4.7 T. M. J. Quast*, G. Ward*, R. Treaff*, T. A. Kent* (sponsor: changes is under investigation. observed to accumulate over time, first in cortical regions, and clearly demonstrated lesions in rats: 1) Before and after death each pixel from this and a spin echo image (T_E 40, T_R 1000) occurred from signals originating at 1.3 ppm in the proton becoming visible on standard MRI. The mechanism of these changes is under investigation.


In magnetic resonance imaging (MRI) and spectroscopic studies of the brain in the immediate post-arterial occlusion period, we noticed that heavily T2-weighted images did not demonstrate differences for several hours while a perfusion deficit could be clearly seen (Kent, et al., AJNR, in press) and lactate and high energy phosphates were altered within a few minutes (Bradley et al., AANS, 1981). We have pursued other methods to image early changes. Studies indicate that fatty acid levels are increased in ischemia (Rehacek et al., Neurochem, 1982). Phase contrast imaging (Dixon, Radiology, 1984) provides a method to separate fat and water signals. We optimized the 180 refocusing pulse time shift so that major changes occurred from signals originating at 1.3 ppm in the proton NMR spectrum. Difference images, obtained by subtracting each pixel from the baseline obtained with a spin echo image (TE 40, TR 1000) clearly demonstrated lesions in rats: 1) Before and after death by anesthetic overdose, in which increases in signal were observed to be most pronounced in cortical regions, and 2) focal ischemia by MCA occlusion, 3 cerebral vessel and single carotid artery occlusion, when lesions occurred prior to becoming visible on standard MRI. The mechanism of these changes is under investigation.


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CEREBRAL METABOLISM AND BLOOD FLOW III

399.3


Neuronal activity is a major factor regulating intercellular K+ (K+ic) and 1CBF. In previous studies, a potassium concentration of K+(K+) from neuronal activation was thought to influence ICBF by producing cerebrovasodilation. Recently, however, it was observed that neuronal activation may also reduce ICBF by producing cerebroconstriction. This study was performed to determine the role of neuronal activation on cerebrovascular responses. Five adult Sprague-Dawley rats were used in a double-blind, placebo-controlled study. The rats were subjected to an isoflurane-induced whole-body vasodilator challenge. 1CBF was measured using the H2 washout method. A 10% reduction in 1CBF was observed. This reduction was due to an increase in resistance, as isoflurane has no effect on cerebral resistance vessels. Therefore, we are statistically analyzing glucose utilization rates and comparing the same time points and magnitude to a rise in [K+] or (presumably) astroglial [K+]. Hence, the "coupling" between functional activity & ICBF is not based solely on changes in interstitial or astroglial [K+].

399.4


Cortical blood flow (CBF) was measured during serial seizures in rats with open or intact skulls to rule out possible time and attenuation of ictal increases in CBF results from hemodynamic changes in the open cranium. Seizures were induced at 6-10 min intervals with pentyleneetrazol in anesthetized, paralyzed rats, and CBF was measured bilaterally by H2 washout. Mean CBF early in the series and diminished to 120-150% over control during later seizures, regardless of whether the skull was open or intact or whether measurements were made in the right or left cortices. Although exposing the cortex results in overestimation of CBF values with the H2 washout method, probably due to diffusion of H2 into the atmosphere, it does not alter the percent increases in CBF measured during serial seizures. (Supported by NIH grant NS-17443.)

399.5

MODIFICATION OF CEREBRAL HYPERMETABOLISM AFTER CORTICAL SEIZURE. EXPRESSION OF GLUCOSE UTILIZATION RATES AND CYTOCHROME OXIDASE HISTOCHEMISTRY. M. A. McDonald, F. M. Rollins, E. A. Queen, and D. M. Finney. Deps. of Psychol. and Physiol., GMU, Albuquerque, NM 87131.

A widespread reduction in cerebral oxidative metabolism, demonstrated by cytochrome oxidase histochrometry (COH) has been reported at 48 hr following a single kindling convulsion in the rat. The present study investigated the expression of glucose utilization by autoradiographic 2-Deoxy-D-Glucose (2-DG) and COH in the same brain areas remote from the injury. This parametric study further examined effects of right hemisphere cortical stimulation (RSCS) on the rat brain utilizing both autoradiographic 2-deoxy-D-glucose (2-DG) and COH procedures. At 48 hr post-SHC, or sham surgery, rats received either 2 mg/kg MPAH or saline and at 24, 48, and 16 days post-injury, the 2-DG procedures were repeated. Autoradiograms were quantified by computer-assisted procedures. At 24 hr post-SCC or sham surgery, rats were anesthetized, ventilated and perfusion fixed with a sodium carbonate-glutaraldehyde buffer. Before Ba2+ superfusion (110 ± 0.1 mM), 2-DG was applied and autoradiograms obtained. In the sham and control groups, 2-DG uptake was not significant. However, in the experimental group, 2-DG uptake was significantly decreased in the same brain areas remote from the injury. Therefore, we are statistically analyzing glucose utilization rates and comparing the same time points and experimental manipulations also using CM of COH.

399.6

MAINTENANCE OF CEREBRAL METABOLISM DURING STIMULATION OF THE FASTIGIAL NUCLEUS IN RATS: D. M. Patteckas, J. L. Williams, D. D. Heistad and N. T. Tamman. Lab. of Neurobiology, VANC and Univ. of Iowa, Iowa City, IA 52242.

Autoregulation studies in the cat have suggested that electrical stimulation of the rostral fastigial nucleus (rFN) increases cerebral blood flow (CBF) without changing metabolism and impairs autoregulation. In contrast, using the pulsed Doppler and microsphere techniques in the cat, we have found only minimal changes in CBF during stimulation of the rFN. We have used microspheres to reassess the effects of rFN stimulation on CBF in the rat. In 7 chloralose-anesthetized rats, CBF was measured during a control period and during electrical stimulation of the rFN. Arterial blood pressure was controlled and stimulus sites were confirmed histologically. Mean arterial pressure increased from 85 ± 5 to 109 ± 5 mmHg during stimulation (P < 0.01). Renal blood flow (in ml/100 gm/min) decreased from 691 ± 104 to 546 ± 100 (P < 0.01) while myocardial blood flow increased from 375 ± 36 to 579 ± 50 (P < 0.01). CBF did not change significantly (from 83 ± 13 to 99 ± 16) rCBF did not change significantly (from 83 ± 13 to 99 ± 16). Post-stimulation, CBF was measured during a control period and during electrical stimulation of the rFN. Arterial blood pressure was controlled and stimulus sites were confirmed histologically. Mean arterial pressure increased from 85 ± 5 to 109 ± 5 mmHg during stimulation (P < 0.01). Renal blood flow (in ml/100 gm/min) decreased from 691 ± 104 to 546 ± 100 (P < 0.01) while myocardial blood flow increased from 375 ± 36 to 579 ± 50 (P < 0.01). CBF did not change significantly (from 83 ± 13 to 99 ± 16). Cerebral vascular resistance increased by a mean of 9% (NS). This study suggests that stimulation of the rFN has a minimal effect on CBF during a moderate pressor response. The data 1) do not support the hypothesis that the rFN has important effects on CBF and 2) suggest that autoregulation is maintained during rFN stimulation. Supported by HL32205, NS25621, and HL43388.
We have tested by histological studies that the volume, surface area, and percentage of perfused capillaries varies among CNS structures. The experiments were performed on awake Sprague-Dawley rats. Regional cerebral blood flow (rCBF) was measured using radioactive autoradiography, and morphometric analysis of light micrographs. The following parameters were assessed: 1) local cerebral blood flow, 2) plasma, red cell, and blood volume of perfused vessels (PLM), and 3) transfer rate constants and permeability-surface area (PS) products for several radiotracers, and 4) total capillary volume fraction (Vc) and surface area (Sa). Among the brain areas examined were the suprachip nucleus (SON) and ventromedial nucleus of the hypothalamus (VMH).

Based on the ratios of blood volume of PLM to capillary volume fraction, the volume percent of perfused-labeled microvessels, which are probably underestimated by this approach, ranged from 64% (SON) to 95% (VMH). Comparisons of PS products to total capillary surface area suggested that the percentage of surface area perfused is least in the SON (about 35%) and greatest in the VMH (100%). Although the capillary volume and surface area percentages were somewhat different for the individual structures, the order from lowest to highest percentage was the same by both measures and supported the working hypothesis.

We have proposed and tested the hypothesis that the volume, surface area, and percentage of perfused capillaries varies among CNS structures. The experiments were performed on awake Sprague-Dawley rats. Regional cerebral blood flow (rCBF) was measured using radioactive autoradiography, and morphometric analysis of light micrographs. The following parameters were assessed: 1) local cerebral blood flow, 2) plasma, red cell, and blood volume of perfused vessels (PLM), and 3) transfer rate constants and permeability-surface area (PS) products for several radiotracers, and 4) total capillary volume fraction (Vc) and surface area (Sa). Among the brain areas examined were the suprachip nucleus (SON) and ventromedial nucleus of the hypothalamus (VMH).

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We tested the hypothesis that GCs modulate the effect of plasma epinephrine on regional cerebral glucose utilization (rCMRgl). Rats were divided into four groups: 1) adrenalectomized (ADX) three days prior to the experiment; 2) intact; 3) intact + adrenals; and 4) intact + one group of epinephrine-infused rats. Plasma glucose decreased as a result of ADX but was increased in both ADX and I groups that received epinephrine. Elevated plasma epinephrine had no effect on rCMRgl in the rats with intact adrenals. However, in the ADX rats, iv infusion of epinephrine increased rCMRgl in every brain region measured (13 of 17 regions statistically significant). Since the response to epinephrine was different depending on the presence or absence of GCs, we conclude that GCs can modulate the effects of plasma epinephrine on rCMRgl.

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Acute renal failure in humans and rats engenders a uremic syndrome of progressively deranged serum chemistry and neurobehavioral deficits. There is reduced EEG power with increased visual evoked potential latency. It is believed that the syndrome is engendered by the neurotoxic effects of a dialytically removable uremic solute. To survey the central metabolic correlates of the phenomenon, rats were surgically implanted with chronic ECG electrodes, a peritoneal dialysis (PD) catheter, and a jugular catheter. Nephrectomised bilaterally (NX), these animals were assigned to the following treatments: (A) attrition, n=7, dialyzed as above; (B) control, n=10, sham operated, undialyzed. We find that in animals sacrificed 48 hours after NX regional cerebral glucose uptake (rCGU) and the EEG implicating this solute in uremia's neurotoxic effect.

INVERTEBRATE MOTOR FUNCTION

400.1 DEVELOPMENTAL CHANGES IN POSTSYNAPTIC NEURONS CAUSE LOSS OF A MONOSYNAPTIC REFLEX DURING METAMORPHOSIS IN MANDUCA SECTA. J.C. Weeks and G.A. Jacobs. Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720.

Manduca larvae exhibit a proleg withdrawal reflex mediated by direct synapses between proleg hair afferents and the proleg retractor motorneuron PPR. During the 4 th larval-pupal molt, ecdysteroid hormones cause PPR's dendritic arbor to regress substantially. Over the same time course the affenter-evoked excitation of PPR is severely attenuated; the compound EPSP evoked in PPR by a shock delivered to the sensory nerve is 70% smaller in pupae than in larvae. PPR's input resistance does not change significantly during this period. This decreased synaptic efficacy could be due to pre- and postsynaptic factors. The afferents change minimally during the larval-pupal transformation, suggesting that postsynaptic changes (e.g., PPR's regression) might be sufficient to cause loss of the reflex. This hypothesis was tested by using hormone treatments to generate heterochronic mosaic pupae which retained a larval proleg. In mosaic hemisegments the afferents remained in the larval state, while PPR repressed normally. The strength of the sensory-to-motorneuron pathway was attenuated to the same degree in mosaic hemisegments as in normal pupae. Thus, the loss of the withdrawal reflex appears due to developmental changes in postsynaptic rather than presynaptic neurons. Supported by NIH & Sloan grants to JCW, and an NIH-NRSA fellowship to GAJ.

400.2 DEVELOPMENTAL CHANGES IN PRE-ECIDYSIS MOTOR PATTERNS OF THE MOTH MANDUCA SECTA. C.I. Miles and J.C. Weeks. Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720.

Manduca exhibits a pre-ecdysis behavior just before ecdysis, which the olfactory centers of the antennae. At the larval-pupal molt, both pre-ecdysis and ecdysis behaviors are triggered by the peptide eclosion hormone (EH; 1, 2). Pre-ecdysis behavior is characterised by rhythmic movement of the abdomen which are robust at molts between larval instars, but are no longer apparent at the larval-pupal molt. The only rhythmic movements at this time are weak dorsoventral flexions of the anteriormost abdominal segments. We have identified this behavior as a weakened version of larval pre-ecdysis by its timing, its dependence on EH, and the firing patterns of the motorneurons (MNs) which produce it. The strength of the motor pattern and the synaptic drive to preganglionic MNs are greatly reduced at the larval-pupal molt. This is unlikely to be due to dendritic regression of the MNs, as they do not show significant structural changes at this time. Furthermore, the activity of these MNs during ecdysis, which immediately follows pre-ecdysis, is as robust as at larval molts. Thus, changes in the structure and/or functions of interneurons presynaptic to the pre-ecdysis MNs appear responsible for the weakening of this motor pattern at the larval-pupal molt. J. Copenhaver & Truman '82, J. Insect Physiol. 28:695; 2,Truman et al. '81, Nature 291:70. Supported by an NIH fellowship to CIM and NIH and Sloan grants to JCW.

400.3 DESCENDING NEURONS RECEIVING COMMON SENSORY INPUTS DIVERGE FROM THE INSECT BRAIN TO FUNCTIONALLY DISTINCT MOTOR NEURON POOLS IN THORACIC SEGMENTS. Nicholas J. Strausfeld and Jürgen J.M. Weyertal 112, D-5000 Cologne, FRG.

In Calliphora, intracellular fills reveal a variety of uniquely identifiable descending neurons (DNs), each distinguished by a characteristic dendritic morphology in the brain and by the morphology of its axon collaterals and terminals in segmental ganglia. Dendrites of specific DNs are structurally and functionally isolated into clusters (DN-clusters), each foraminating the core of a discrete neuropil. A given DNC receives a characteristic set of primary mechanosensory afferents, terminals of visual interneurons, and interneurons derived from olfactory centers of the brain. Local, lateral and lateralateral interneurons appear to connect different DNs in a fashion similar to interneurons linking groups of motor neuron dendrites in the thoracic ganglia. Although the same context-specific visual and mechanosensory stimuli can elicit responses from more than one member of a DNC, the axons of these neurons project to different targets. For example, axons from one known DNC diverge to functionally distinct groups of motor neurons belonging to the leg or the flight motor. Usually, however, the axon of any one DNC gives rise to segmental collaterals which appear to innervate motor neuron pools supplying generally homologous muscles. Examples are: branches to pro-, meso-, and metathoracic leg muscles motor neuron branches or branches to pro- and mesothoracic motor neurons supplying anterior dorsal neck muscles and direct flight muscles, respectively. Supported by NIH Grant No. R01 EYO7151-01

400.4 MOTOR INNERRATION OF THE LOBSTER'S WALKING LEG MUSCLES. Theodore J. Wiens, Department of Zoology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The recent finding (Wiens, J. Neurobiol. 16, 183, 1985; Wiens and Rathmayer, J. Comp. Physiol. 156, 305, 1985) that the common inhibitor (CI) innervates all muscles in the walking legs of crabs and crayfish prompted a test of the same hypothesis for the much-studied Homarus americanus. Paired intracellular recordings were made from different combinations of muscles during stimulation of excitatory and inhibitory axons in various nerve branches of the leg. It was found that stimulation of CI in the effenter nerve entering the closer muscle can elicit JPF's in all nine muscles distal to the basipodite, as the hypothesis predicts (comp. Wiersma and Ripley, 1952) - but only if the leg nerve is intact distal to the coxa. Investigation of activation thresholds, JPF latencies, and effects of section suggested that CI splits into two branches in or proximal to the basipodite; one of these supplies the extensor, bender, closer, and opener muscles in that order, and the other the reducer, flexor, stretcher, and rotator muscles. CI's two branches are thus disjioned in the autozomised leg. The rotator's only other effenter innervation comes from one of the flexor muscle's excitatory axons (comp. Sherman and Atwood, 1971). The opener receives a second innhibitory input from the well-described specific opener inhibitor (OI). These results resolve some questions about leg control and raise others.

I thank C.K. Govind for discussions and equipment loans.
INVERTEBRATE MOTOR FUNCTION

400.5  CRAYFISH SWIMMING: DIFFERENT EMG PATTERNS IN ROstral AND CAUDAL ABDOMINAL SEGMENTS. J.Paul and R., Proc. Biol. Soc., Canada (1987). The swimming (nongiant tailflipping) in crayfish relies on a central pattern generator (CPG) whose nature is poorly understood (Reichert et al., 1981). Previous analyses of emgs from swimming crayfish and squat lobster found no correlation between extensor burst latency and extensor burst period (EBP) in rostral segments (52–41), but 55 FBLs in squat lobster covery with EPBs (Wilson & Paul, 1989). We have now found that also in crayfish S5 FBLs and EPBs covery while concurrent S3 FBLs vary inversely with EPBs (Fig). Preliminary results suggest that intersegmental coordination varies with frequency and that this arises from differences in timing of extensor bursts, flexor bursts being nearly synchronous in S3 and S5 at all frequencies. Thus nongiant tailflipping motion is similar in crayfish and squat lobster. The underlying CPG appears to be fundamentally different than CPGs for locomotion using limbs in which similar segmental motor patterns are produced by coupled segmental CPGs.

400.6  THE NEURAL BASIS OF LIGHT-INDUCED WALKING IN CRAYFISH. T.W. Simon and D.H. Edwards, Dept. of Biology, Georgia State University, Atlanta GA 30303.

The backward walking response of crayfish to illumination of the Caudal Photoreceptor neurons (CPRs) in the 6th abdominal ganglion persists in restrained upside down animals. Leg movements in this position were sufficient to turn a walking wheel, and the responses appeared qualitatively similar to normal walking in the same light stimulus. A64 motoneurons are excited when ventrally illuminated. A cephalic response of Tonic Flexor (TF) motoneurons associated with backward walking (Kovac, 1974a,b; Moore & Larimer, 1987) commenced simultaneously with leg movements. High frequency activity in a single CPR, similar to its light response, excited cells in the abdominal nerve cord which are part of a pattern initiating (PI) network for the TF response. When stimulated, some of these neurons could evoke a portion of the cephalic motor pattern; however, none were sufficient to elicit the entire TF response.

Interneuron A64 (Wine & Krarne, 1982), an identified mechanosensory interneuron, is also excited during the backward walking response. A64 is excited by unknown cells in the rostral portion of the abdominal nerve cord and may contribute to their excitation during the walking response. In addition, swimmeromotor neurons and some hitherto unidentified cells which may contribute to their excitation were excited during the PI. Both walking and the TF response to 6th ganglionic illumination persist following ligation of both circumesophageal connectives, and we are attempting to localize where the CPRs provide input to neurons which initiate walking.

400.7  EXCITATION AND INHIBITION OF THE CRAYFISH SWIMMERET RHYTHM BY STIMULATION OF SECOND NERVES OF THORACIC GANGLIA. A.Chrachri* (SPON: H.Anderson) Dept. of Zoology, Univ. California at Davis, Davis CA 95616.

In the crayfish, Pacifastacus leniusculus, the swimmeret motor pattern is strongly modified by tonic stimulation of the second nerves of each thoracic ganglion. Stimulation of these nerves at low intensity provokes a long-lasting activation of the swimmeret motor pattern. The stimulation increases the frequency of spikes of certain motoneurons within bursts in the swimmeret motor pattern, and also increases the amplitude of their membrane potential oscillation. At high intensity of stimulation of the same nerves inhibits the swimmeret rhythm. This inhibition is partially blocked by phenolamine, a competitive blocker of octopaminergic inhibition in the swimmeret system.

Blocking chemical synapses in these thoracic ganglia with low Ca ++, high Mg ++, saline does not block these effects of second nerves stimulation, and Ca ++ backfills of thoracic second nerves reveal neurons in abdominal ganglion 1. Therefore, I suggest that these effects involve cell(s) which are located in ganglion 1, and that they act via pathways within the CNS, not via the blood. From the duration of their effects, these neurons might act through a modulatory process.

Supported by NSF Grant BNS 87-19397 to B.Mulleoney.


Tonic stimulation of individual neurons in the ventral lateral margin (VLM) of the crayfish abdominal nerve cord evokes a long-lasting discharge of abdominal tonic flexor (TF) motoneurons that is part of a backward walking response (Kovac, 1974, Moore & Larimer, 1987). We have found that stimulation of single VLM neurons, including A64, inhibits the LG command neurons for tailflip while they excite the TFs.

The VLM neurons inhibit LG by tonic depolarization. LG EPSPs evoked by sensory root stimulation are reduced in proportion to the level of LG tonic depolarization. LG EPSPs evoked by A64 are also reduced during the tonic depolarizing phase of the LG EPSP evoked by sensory root shock. Since many of the synapses onto LG are electrical, it is likely that LG inhibition results from the postynaptic depolarization itself, potentially by increased LG membrane conductance or by back-biasing rectifying electrical synapses onto LG.

A64 produces a phasic burst followed by an 80 ms, high frequency (up to 300 Hz) spike train in response to phasic stimulation of the abdomen (Zucker, 1972). The phasic burst helps excite LG through monosynaptic electrical synapses. Our results suggest that the tonic phase of the response acts to inhibit LG by tonically depolarizing it. LG EPSPs from additional inputs would be reduced during this period, which coincides with recovery from the initial tailflip.

400.9  THORACIC OUTPUT OF CRAYFISH GIANT COMMAND NEURONES. M.J. Heitler and K. Franson*. Gotby Marine Lab, Univ. St. Andrews, Scotland, KY16 8LB.

The crayfish escape tail-flip is initiated by 2 sets of giant command interneurons, MG and LG. The abdominal circuitry driven by these neurons has been extensively studied, but little is known about their thoracic output. We describe the anatomy and physiology of 3 identified segmental neurons in the 4th and 5th thoracic ganglia which receive direct input from the giant neurons.

1. Leg Promotor Motoneuron. This is driven 1:1 by the MG through a rectifying electrical synapse. It has powerful excitatory neuromuscular output, which shows no increases in tonic burst latency as followed by slow facilitation. This is very similar to the output of the abdominal motor giant neuron.

2. Segmental Giant Motoneuron. This is driven 1:1 by the LG (TG5) and LG and MG (TG4) through rectifying electrical synapses. Its axon terminates close to the ganglion in numerous fine branches, which are located entirely within the nerve root. The LG drives fast flexor motoneurons of the trunk musculature.

3. Motor Giant Motoneuron. This is driven 1:1 by the LG through a rectifying electrical synapse. All three neurons receive depolarizing IPSPs which can inhibit their input from the giant neurons. The significance of these neurons will be discussed in relation to the possible evolution of the escape from a limb-driven to a trunk-driven behaviour.


We recently reported that mechanosensory stimulation of a swimmeret in lobster produces tonic abdominal extension (J. Comp. Physiol.115:325,161:695;J. Neurobiol.17:421). This includes excitation of the flexor inhibitor f5 and extensor excitor motoneurons and suppression of the extensor inhibitor e5 and the flexor excitor motoneurons. The present work characterizes interneurons that both drive (or inhibit) posterior motoneurons and receive inputs from swimmeret mechanoreceptors. Flexion producing interneurons (PIs) when current injected excited the flexor extor motoneurons. PI stimulation was inhibited by mechanostimulation of feathered hairs, smooth hairs and integumentary receptors in the swimmeret, suggesting that PI suppression by swimmers in generating flexion inhibition/extension activity. However, since afferents are never inhibitory, the neural circuit for such responses must include at least one layer of interneurons between the afferents and PIs. Extension producing (PIs) when depolarized by current injection drive f5 and inhibit the flexor exciters. Since there was no 1:1 phase relationship between interneuronal and motoneuron responses to mechanostimulation of the swimmeret, a multisynaptic pathway between swimmeret afferents and tonic motoneurons must exist.
400.11
CHOLINERGIC / SEROTONERGIC SENSORY NEURONS HAVE BOTH CLASSICAL AND MODULATORY SYNAPTIC EFFECTS ON NEURONS IN THE CRAB STOMATOGASTRIC GANGLION. P.S. Katz, M.H. Figur, a and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

We have found a novel receptive cell type in the stomatogastric nervous system of the crab, Cancer borealis, that not only provides fast synaptic input, but also has longer lasting modulatory effects on the burning properties of central pattern generator neurons. We previously reported the existence of the GPR cells, a set of 4 muscle receptor cells in the gastro-pyloric region of the crab stomach (Katz and Harris-Warrick, 1987). These cells contain serotonin as determined by immunohistochemical staining and HPLC (Beitz et al., J. Biol. 109:35, 1984). We now show that they also have choline acetyltransferase activity, suggesting that serotonin and acetylcholine are co-transmitters in these cells. The GPR cells produce an array of rapid synaptic effects in different stomatogastric ganglia, including EPSPs of different time courses that are pharmacologically blocked by nicotinic antagonists. In addition, GPR activation causes long-lasting modulatory effects in specific neurons, such as the induction of burning and plateau potentials. These effects accumulate with repetition, and can outlast the duration of the stimulation by more than a minute. The GPR cells thus provide more than just a cycle-by-cycle correction of the motor output. Our results suggest that by altering the physiological state of some of the component neurons for extended periods of time, sensory input to central pattern generators can play a modulatory and instructive role.

(This work was supported by NIH NS 17323 and Harc-NYC-191410.)

400.13

Intersegmental interneurones in a mesothoracic population receive mechanosensory inputs from a middle leg (Laurent, G. 1987 J. Neurosci. 7:2957–2969). They project laterally to the metathoracic ganglion where they make output connections (87% excitatory, 13% inhibitory) with mesothoracic intersegmental interneurones. These non-spiking interneurones can gate or modulate metathoracic modulatory and instructive role.

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400.14

Insects have often been represented as ideal poikilothersms, yet they are able to survive and function over a wide range of temperatures. It has generally been assumed that changes in behavior from temperature effects are related to changes that occur in neural processes. Previous investigations have shown that changes in temperature affect the conduction velocities of nerve fibers, as well as the length-potential patterns of activity that arise in higher centers. The way in which the output of the nervous system compensates for these induced changes in input, however, is not well understood. The present work focuses on the effects that ganglion temperature has on the neural activity in the dragonfly. Spontaneous extracellular recordings in the metathoracic ganglion were obtained from both individual cells and groups of cells over a range of approximately 25°C. Spike frequency and spike slope were characterized for individual large cells. Intraspineal and extracellular histograms were obtained as a measure of total neural activity. Results indicated alterations in large cell spike frequency and slope as a function of temperature change.

Changes in small cell activity were not as pronounced. Notably, the overall pattern of total neural activity remained relatively constant. The possible significance that temperature induced changes in spike slope may have on regulatory neural output is discussed.

400.15
A BIOMECHANICAL ANALYSIS OF THE HEAD WAVING RESPONSE IN APLYSIA. F.M. Kupfer and T.J. Carew. Dept. of Biology, Yale Univ., New Haven, CT 06520.

The head waving response (HWR) in Aplysia is a complex behavior that can be modified by opsin condition (Cook and Carew, 1984, 1987). An analysis of the HWR and its plasticity requires an understanding of how the response is mechanically produced.

The Aplysia body is a soft, constant-volume container, consisting of a muscular wall filled with a fluid (hemocoel). Thus biomechanically, movement can be produced either by: 1) simple contraction and relaxation of appropriate muscles, or 2) contraction coupled with modulation of hydrostatic pressure. We investigated the involvement of hydrostatic mechanisms in freely moving animals by cannulating their hemocoel and monitoring pressure with a solid state transducer. The basal pressure of resting animals was 1–2 cm of water and its modification by learning.

400.16
MODULATION OF APLYSIA RENAL PORE ACTIVITY BY L10, THE LU CELL AND AN IDENTIFIED PERIPHERAL MOTONEURON. K. Koschier, Center for Neurobiology & Behavior, NY State Psychiatric Institute, and Columbia University, New York, NY 10032.

Renal excision in Aplysia begins with the filtration of blood across the wall of the heart. This ultrafiltrate is swept from the pericardium into the renal sac through a ciliated renal pore that is located near the posterior base of the gill. The urine leaves the renal sac via the muscular renal pore near the posterior base of the gill. The renal pore, which is located in the abdominal ganglion, increases heart rate by inducing heart activity and the C cell, which is the motor neuron for L10, activates the renal pore. The L10 cells are identified by their location on the gill and their spiking pattern.

We have previously shown that L10 excites the radial (opener) muscle of the pore and inhibits the circular (closer) muscle. When L10 fires, the opener muscle fibers generate a synaptic action potential that does not follow the L10 spikes 1:1. Each time a twitch is generated, four events can be discriminated in an extracellular recording from the pore. These events are: (1) L10's axon spike, (2) the muscle junction potential due to L10's direct connection to the opener muscle. (3) L10's large muscle action potential. Events (1) and (2) occur every time that L10 fires. Events (3) and (4) occur only when L10 fires, and they always occur together. We have recorded these events as a single peripheral motoneuron that is embedded in the lateral side of the renal sac. Oxy injections show that this bipolar peripheral neuron, called the LP cell, is completely surrounded by the innervation patterns of L10.

We have also identified that a subset of these cells inhibits the twitch that occurs during pore opening. We have identified that they also block events (3) and (4). Current work addresses the issue of whether the inhibition of the RP cell by the LP cells is pre- or post-synaptic in origin. The RP cell provides a good voltage point of which to study the interaction of the central and peripheral nervous systems of Aplysia.

(Supported by NIH grant NS14385.)
Inembetrate motor function


Medial gastrocnemius (MG) motoneurons were retrogradely labeled with horseradish peroxidase (HRP) following soak of the muscle nerve in rats of various age groups. Limb-section of spinal cord was sectioned horizontally and reacted with tetramethyl benzidine. The cell bodies and proximal dendrites of heavily labeled motoneurons were reconstructed.


Research on early neurobehavioral development of movement (motility) patterns has revealed the existence of "cyclic," spontaneous movement patterns in a variety of species. Cyclic, spontaneous movement patterns have been described prior to birth or hatching, and after birth, and range in frequency from 1-3 minutes per cycle. However, there are questions concerning the locus of neural (brain and/or spinal cord) regulation of the cyclicity, and questions regarding the degree to which the movements themselves are "random" or "organized (frequently recurring)." The present study used newborn rats and a computerized movement analysis system to quantify 12 characteristics of spontaneous movement (e.g., mouth, head, limbs, trunk, posture change, ambulation). The first spontaneous movement of the newborn rat was found to share a common cycle frequency (range: 0.5-1.0 cycles/min) for all body segments measured, suggesting a "cyclic" generator in the brain and spinal cord. However, preliminary spinal transection data indicate a brain cyclic generator, based on the loss of movement cyclicity caudal to the transection in newborn rats. The overall results also suggest that the spontaneous movements of newborn rats may be organized, and not random as previously thought. (NIH Animal Care Guidelines followed.)


Rats prenatally exposed to ethanol have more corticospinal projection neurons than controls (Miller, J.C.N. 222:372, 1987), hence, we examined the number of pyramidal tract axons in rats examined at 10 days postnatally. The density of myelinated and non-myelinated axons was similar in the two groups. Axons were smaller and the myelin was thinner in Et-treated rats than in controls. The estimated number of axons in the pyramidal tract at the level of the lower lumbar enlargements was 10% smaller in Et-treated rats.

401.4 INTRACELLULAR STUDY OF CORTICOSPINAL PROJECTION NEURONS IN RATS PRENATALLY EXPOSED TO ETHANOL. W.H. Miller, F. Klung, N. Chiaia, and R.B. Rhodes, Department of Anatomy, School of Osteopathic Medicine and Robert Wood Johnson Medical School, UMOM, Piscataway, NJ 08854.

The effects of prenatal exposure on the structure and function of corticospinal projection neurons in mature somatosensory cortex of rats examined at 10 days postnatally were characterized via intracellular recording techniques. Corticospinal neurons were identified as cortical neurons antidromically driven by the stimulation of either the contralateral or ipsilateral corticospinal tract. Individual corticospinal neurons were injected intracellularly with horseradish peroxidase (HRP). Rats were sacrificed and the tissue was processed by standard HRP histochemical techniques. All intracellularly labeled neurons were pyramidal neurons. In Et- and Ch-treated rats, the cell bodies of these neurons were distributed in layers II/III and VI as well as layer V. Shell analyses of the body of the corticospinal neurons in layer V showed dendritic trees were significantly (p<0.01) more complex and more extensive in Et-treated rats than in controls. The conduction latency was significantly (p<0.05) shorter in Et-treated rats than in controls. These results suggest that ethanol exposure in utero alters the structure and function of corticospinal neurons.

Support by NIH grant HD22703.
401.5
TRANSPLANTS ALTER THE DEVELOPMENT OF SENSORIMOTOR FUNCTION AFTER NEONATAL SPINAL CORD DAMAGE. E. I. Tolbert, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA.

401.6

401.7
ACTIVITY OF CORTICAL NEURONS WITH TRANSIENT COLLATERAL PROJECTIONS TO THE CEREBELLM. D. L. Tolbert, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA.

401.8

401.9
THE MATURATION OF AFFERENT PROJECTIONS TO THE RAT CEREBELLM. N. J. Valenzuela*, C. Grayzel (a), N. Leclerc (a), L. Elsegard (a), T. Blais (a) and N. Adams (a).

401.10

401.11
STIMULATION OF EMBRYONIC CHICK IN OVO: TECHNIQUES FOR FOCAL ELECTRICAL STIMULATION OF BRAINSTEM LOCUS COERULUS. G. C. Lucas, Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

401.12
Our studies were aimed at examining neurophysiological parameters associated with spinal cord injury-related motor impairment in a rat model which previously has been characterized by indices of behavior and anatomy.

401.13
Contusive injuries were produced using a modification of the Allen weight drop method and motor performance was assayed by the combined behavioral scoring (CBS) method of Wrathall and coworkers. Spinal reflex excitability was tested utilizing individually recorded H-reflexes. The extent of the spinal lesions was quantified by histological analysis. At one week post-injury we have observed that animals exhibited mean CBS scores of 74.0 (s.d. = 22.0; n = 5). Reflex amplitudes were not significantly different from controls (4.4 M ratio: 0.57, s.d. = 0.04; n = 5 compared to 4.4, s.d. = 1.56, n = 8). However, the sensory input required to achieve maximal reflex amplitudes in the injured animals was significantly less than in the normal animals. In addition, recovery curves of muscle and skin afferent-evoked reflexes suggested that presynaptic inhibition of reflexes in the injured animals was significantly reduced compared to intact controls.

401.14
These studies suggest that motor impairment occurring within one week after contusion-injury of the rat spinal cord is most closely correlated with interactions that depend upon interneuronal changes in primary afferent and motoneuron excitability. (Supported by NINCDS No 1-NR5-7-2300).
HINDLIMB MUSCLE SYNERGIES IN SPINAL TRA NS SECTED CHICK EMBRYOS. D.J. Sandstrom and J.C. Weeks. Graduate Group in Neuroscience and Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720. In Manduca, the physiology, anatomy and developmental fate of many motorneurons (MNs) are known, whereas virtually nothing is known about interneurons (INs) projecting to MNs. An isolated, dehydrated ganglion preparation has greatly facilitated recording from small INs and has allowed pairwise recordings from INs and MNs. A number of MNs appear to have a single target cell in the ganglion, allowing identification upon recording. Following injection of the ON-inhibitory cell, the MNs were identified by their response to stimulation of the corresponding motor nerve. The postembryonic development of these INs is currently under investigation. Supported by NSF fellowship to DJJ and NIH and Sloan grants to JCM.

ISOPYCNIC CENTRIFUGATION EMPLOYING PERCOL STEP GRADIENTS FOR THE PURIFICATION OF MOUSE FAST AVOIDANCE BEHAVIOR NEURONS FROM SPINAL CORD HONGUEXETINS. MDStrong, RN. Gurrola and G. Gaduse. Lab. of Central Nerv. System Studies, Natl. Inst. Health, Bethesda, MD 20892. We have developed a technique employing the principles of isopycnic centrifugation for the rapid isolation of a cholinergic cell population from fetal spinal cord homogenates for studies of neurofilament catabolism in motor neurons. Pooled spinal cord tissue from 19 to 21 day fetal mouse embryos (E19.5-21.5) are dissociated, the suspension layered onto discontinuous Percol step gradients (densities 1.040, 1.050, 1.060) formed in polycarbonate tubes and centrifuged at 800 rpm at 4°C in a Sorval swing-out rotor. The distinct cell populations collected at each interface can be directly plated without a wash step, enhancing yields and viability (85-95%). Data will be presented to demonstrate the separation of cholinergic neurons from non-cholinergic cells.

IDENTIFIED INTERNEURONS IN LARVAL AND PUPAL ABDOMINAL CYCLES/EPODE THAN IN INTACT EMBRYOS. R.A. Volvo and M.H. Droogs. Dept. of Biology, Texas Woman's Univ., Denton, TX 76204. Our objective is to use long-term explant cultures of spinal tissue to establish a model system for investigating the cellular/network mechanisms of motor pattern generation in mammals. Explants are being conducted on tissue explants and on tissue taken from intact animals to determine how closely these neurons represent in situ ventral horn neurons. Explants of tissue taken from intact embryo development yield a mixed population of predominately motoneurons. The second, transverse sections of lumbar tissue (200 - 500 µm) taken from 13 - 14 day gestation mice have been placed as explant cultures using a roller tube technique as described by Gahrweiler (J. Neurosci. Meth. 4: 329-342, 1981). A minimum of three weeks in culture, tissue explants were removed for identification of ventral horn motoneurons using Karnovsky's acetylcholineesterase (AChE) staining method as modified by Gahrweiler et al. (Lab. Invest. 42: 313-323, 1979). Explants were incubated in the impervious dura mater after perfusion with paraformaldehyde, and incubated in acetylcholine esterase staining solution. A parallel series of experiments was carried out on intact lumbar tissue taken from a littermate on the same day of culturing. Total cholinergic cell counts, cell density measurements and soma size distributions are being compiled using a Zeiss Videoplan Image Analysis System to quantify the cell loss due to the explantation and culturing process. Supported by NIH Grant #1 R29 NS2520-01.

INTERNEURONS INVOLVED IN MULTISEGMENTAL REFLEXES IN LARVAE AND PUPAE OF THE MOTH MANDUCA SEUTA. B.Waldrop and R.B.Levine. ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721. We are investigating the postembryonic development of neural circuits involved in the gnash reflex of Manduca. The gnash reflex is a pupa-specific structure containing mechanosensory hairs which cause the larva to close its mandibles by evoking a powerful contraction of ipsilateral muscles. The sensory neurons, motor neurons and muscles of the gnash reflex are derived from the larval segmental nerves. The sensory neurons and the dendrites of the motor neurons are in different ganglia, and there must be interganglionic interneurons carrying the sensory information. We are investigating the morphology and development of these interneurons and to determine if they are also retained and modified during metamorphosis. The experimental preparation consists of an isolated abdominal nerve cord from either a larva or a pupa. Electrical stimulation of the sensory nerve leads to stage-specific response patterns recorded intracellularly from motor neurons. Interneurons are impaled and tested for responses to the sensory stimulus, and their ability to evoke PSPs or changes in the spike rates of the motor neurons in response to electrical stimulation in the larva and the pupa which satisfy both of these criteria. Intracellular cobalt fills have revealed both dorsal and ventral dendrites and interganglionic axons, consistent with a role for these interneurons in sensory-motor integration in the gnash reflex. Supported by NSF BMS 8607066 and NIH #T32 NS07309-01.
IMMUNOLOGICAL REACTIONS IN INTRAVENTRICULAR XENOGRAFTS AND ALLOGRAFTS. Major focus was on whether or not the host immune system is stimulated by xenografts (e.g., human fetal tissue) or allografts (e.g., fetal islets) and the effects on the grafted tissue. We have studied this by intraventricular grafts of human fetal tissue in neonatal and adult recipients. These grafts were derived from human fetuses of various gestational ages and were transplanted into the lateral ventricle of recipient rats. Immunohistochemical studies showed that the grafts were well integrated into the host brain parenchyma and were reactive to host antibodies. In addition, the sequence of events following transplantion of fetal tissue includes the development of inflammatory cells around the graft and the formation of a reactive gliotic response. Immunohistochemical studies also showed that the grafted tissue was reactive to host antibodies, indicating that the grafts were not completely rejecting. However, the extent of this immune response varied depending on the gestational age of the graft. We have observed that the immune response to xenografts is less severe than that to allografts, suggesting that the host immune system is less reactive to xenografts than to allografts.

Nerve growth factor (NGF) was shown to enhance the survival and differentiation of human fetal nerve cells in culture. We have investigated the effects of NGF on the survival and differentiation of human fetal nerve cells cultured in vitro. NGF was added to the culture medium at various concentrations, and the effects on the survival and differentiation of the cells were measured. NGF was shown to increase the survival and differentiation of the cells, and this effect was dose-dependent. The optimal concentration of NGF for the survival and differentiation of the cells was found to be 10 ng/ml. These results suggest that NGF is a crucial growth factor for the survival and differentiation of human fetal nerve cells.

In another study, we investigated the effects of a cholinergic agent, physostigmine, on the survival and differentiation of human fetal nerve cells. Physostigmine was added to the culture medium at various concentrations, and the effects on the survival and differentiation of the cells were measured. Physostigmine was shown to decrease the survival and differentiation of the cells, and this effect was dose-dependent. The optimal concentration of physostigmine for the decrease in survival and differentiation of the cells was found to be 100 ng/ml. These results suggest that physostigmine is a crucial agent for the decrease in survival and differentiation of human fetal nerve cells.

In conclusion, our studies have shown that NGF and physostigmine have significant effects on the survival and differentiation of human fetal nerve cells. These findings suggest that NGF and physostigmine may be useful for the treatment of neural disorders, such as Alzheimer's disease and Parkinson's disease.
402.5
HOST-GRAFT INTEGRATION: RAT PERIAQUEDUCTAL GRAY AND ISOAQUEDUCTAL GRAY IN CHRONIC PAIN REGIONS
J. Sagen, L. Low, and W. Pappas. Dep. of Anatomy and Cell Biology, Univ. of Ill at Chicago, Chicago, Ill. 60612.
Previous work in this lab has demonstrated survival of rat adrenal medullary tissue following transplantation into rats (1). It is important to study the role of these cells in chronic pain modulation. We have shown that adrenal medullary transplants can survive and form connections when transplanted into injured cortex following traumatic brain injury. We examined the ability of fetal cortical transplants to proliferate and survive in the host at different stages of cavity development. Male Sprague-Dawley rats (350-450g) received a stereotaxic injection of whole fetal cortical tissue (E14 to E16) into the left lateral ventricle of the rat brain. The transplants grew to 4x original size and exhibited limited to some extent by these barriers. Furthermore, the transplants are important in mediating this analgesia. The ability of foetal neurones to stably incorporate the BAG retrovirus contains the E.Coli B-galactosidase gene downstream of the E. coli lacZ promoter in the lacZ operon. We have recently used this method to determine the fate and origin of neurones transplanted into the rat brain. In this study, the BAG virus has been used to infect rat foetal striatal neurones before the infected neurones were transplanted into the ibotenate lesioned striatum of the adult rat. Following transplantation, both control tissue and transplanted tissue were assessed for changes in pain sensitivity following adrenal medullary transplantation. The basal levels of met-enkephalin levels in the brain of rats with adrenal medullary transplants were assessed for changes in pain sensitivity following adrenal medullary transplantation. The basal levels of met-enkephalin levels in the brains of rats with adrenal medullary transplants were assessed for changes in pain sensitivity following adrenal medullary transplantation. The basal levels of met-enkephalin levels in the brains of rats with adrenal medullary transplants were assessed for changes in pain sensitivity following adrenal medullary transplantation. The basal levels of met-enkephalin levels in the brains of rats with adrenal medullary transplants were assessed for changes in pain sensitivity following adrenal medullary transplantation. The basal levels of met-enkephalin levels in the brains of rats with adrenal medullary transplants were assessed for changes in pain sensitivity following adrenal medullary transplantation.
302.11  

We are presently evaluating different methods for labeling donor cells prior to neural transplantation in the olfactory system. This investigation offers the possibility of using labeled human or animal spinal cord cells in PHA-L has been reported to allow discrimination of donor cells grafted into pre-lesioned adult rat cerebral cortex (Kapp et al: J. Neurosci. Res. 76:143, 1987). We are incubating solid tissue blocks of rat fetuses of embryonic ages 15-18 days in 1% PHA-L prior to transplantation to determine the integrity of the intact or pre-lesioned neonatal rat olfactory system. In addition to sections reacted for PHA-L by the ABC method, cell and fiber stains are used on alternate frozen sections. The cell and fiber stained sections show "viable grafts" with appropriate neuronal types and with fibers traversing the host-graft interface. However, the PHA-L labeling by this method appears to be incomplete, possibly due to insufficient penetration of the fetal tissue blocks. Further, host neurons at various sites outside of the graft may show PHA-L reactivity perhaps from uptake of label from degenerating donor cells. Use of cell suspensions rather than solid tissue blocks is planned for future studies. (Supported by NIH Grants NS09678 and DE04942. LEW is an affiliate of CDMRC.)

302.13  
FINE STRUCTURAL CORRELATES OF NEURAL TRANSPLANTATION: M. Wu* and D. R. Scott. Dept. of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501. 

This investigation has focused on basic questions of vascularization, survival, and regeneration in neurites in the transplanted endocrine hypothalamus. Normal fetal hypothalamic grafts 15 days post-coitus were transplanted into the ventral, lateral, and medial ventricles of host rats with diabetes insipidus (DI). Blood vessels were extant in grafts within the first week following transplantation. Vasculature within the grafts was from host origin. AVP neurons project fibers which develop three types of neuroanatomical relationships with the host brain: 1) terminations in the host median eminence; 2) terminate around the blood vessels which grow from the host brain into the graft; and, 3) terminate directly into the ventricular lumen of the host brain. Penetrating capillaries with distinctive perivascular spaces were observed in the ventral regions of the graft in proximity to the host median eminence. Four weeks following transplantation, AVP-positive neurons within the graft appeared well differentiated and exhibited numerous axodendritic and axosomatic synapses. This study indicates that the morphological relationships that develop between transplanted AVP-positive neurons and host-brain recipients may allow for AVP-positive neurons to release neuropeptide hormones into both the vascular and CSF compartments. Supported by NSF grant BNS 8709687.

302.15  

In our previous studies involving cell suspension transplants into the dentate granule cell layer, (GCL), it was noted that the fluid volume of the injected cell suspension stippled the host tissue in a manner suggestive of the hippocampal cleavage planes, particularly along the hilus/GCL interface, resulting in axotomy and subsequent death of the granule cell population. We present the observations of this phenomenon and undertake to study the effects of granule cell lesions in the absence of a transplacat. Reproducible GCL lesions were created by stereotactic placement of Barts' Balanced Salt Solution into the GCL of adult rats. After survival times of 6 days to 2 months, brains were analyzed by Tim's stain, histamine and GFAP and S-100 immunoreactivity. GCL lesions resulted in a loss of granule cells, decreased mossy fiber staining thinning of the stratum molecular and a marked gliosis surrounding the lesion. GCL lesions contribute to the altered host environment in transplant studies and provide an important control for studying atrophic responses due to GCL efferents from those of the transplanted tissue. Additionally, specific granule cell lesions can be used to study the function and connectivity of the dentate granule cells. Supported by NS23266.

302.12  

Ardizzoni, Michaels and Arendash (Science 239:635, 1988) have described a method using gold-filled Sendai virus envelopes to mark cell suspensions prior to transplantation. We are examining similarly labeled neural grafts grafted into pre-lesioned neonatal rat olfactory system. Preliminary examination of transplanted labeled tissue in the immediate vicinity of the graft site revealed revealed gold particles associated with various synaptophysin-like membranes of different cell types including neurons, glia and their processes. Unexpectedly, heavy labeling was observed within the nuclei of both neurons and glia. The gold particles vary between 15 and 80 nm diameter and appear as single round profiles or aggregates of several such profiles. While gold label was found throughout the limited area we have examined, its concentration dissipates with distance from the center of the graft. Examination of sites within other grafts, including pre-lesioned target areas is planned. (Supported by NIH Grants NS09678 and DE04942. LEW is an affiliate of CDMRC.)

302.14  

We are investigating immunological correlates of blood-brain barrier (BBB) breakdown in retinal xenografts in rats, utilizing skin grafting to initiate an immune response to the neural transplanted tissue. Embryonic (E13-14) CD-1 mouse retinas were grafted into the brain stem parenchyma of neonatal (P1) Spraque-Dawley rats. Two days after transplantation animals received a 1 cm2 CD-1 skin graft on the flank to provoke an immune response to the neural graft. Control animals received no skin graft. 2-8 days post skin grafting (DPSG) animals were injected in the femoral vein with horseradish peroxidase (HRP). Brains were processed for Nissl, HRP-TMB, and anti-M2, -M6, -lacto, -macrophage, and -astrocyte monoclonal antibodies. Experimental and control animals injected 2-4 DPSG showed no leakage of HRP. A small percentage of 5 DPSG animals showed isolated, patchy leakage, but little or no evidence of rejection. At 6 DPSG over 50% of the grafts showed evidence of HRP leakage and the infiltration of lymphocytes. At 7-8 DPSG massive leakage of HRP and widespread infiltration of lymphocytes, macrophages, and astrocytes was evident. The results demonstrate that the status of the BBB is closely correlated to the immunological status of the host. They also support the presence of a BBB in neural transplants, but suggest that immunological factors should be considered in allo- and xenografts of neural tissue. (NEI 705383 & Winters Fdn)
402.17


Mesencephalon from aborted fetuses is a source of dopaminergic neurons which may be useful for implantation in Parkinsonian patients. A contraindication to the use of this tissue is the high rate of contamination with vaginal flora which prevents a technique to sterilize this tissue in a concentrated antibiotic wash. As a correlative study, we examined possible cytotoxic effects of two concentrated antibiotic solution on cells from fetal mesencephalon. The ventral mesencephalon was dissected from embryonic day 12 or 14 rat embryos were washed either in the concentrated antibiotic solution or PBS with 6% glucose. Cell suspensions were then prepared by papain digestion and mechanical dissociation. Initial viability was assessed by trypan blue exclusion and found to be >80% in both groups. Survival of dopaminergic neurons in cell culture was documented by tyrosine hydroxylase immunocytochemistry. We conclude that exposure of fetal tissue to the concentrated antibiotic solution has no apparent cytotoxic effect.

402.19


Replacement therapy of dopamine in the brain by tumorigenic catecholaminergic cell lines (TCL) requires enclosure and containment of such cells. We studied the survival and the differentiation of tyrosine hydroxylase (TH) of encapsulated TCL. One TCL, the PC12 pheochromocytoma cell line, known to synthesize high levels of dopamine, was encapsulated and implanted in the forebrain of rats maintained in vivo. Cell-filled permissive polymer capsules (Aebischer et al., ASAID 10:96,'87) were fixed at intervals up to six months, the morphology of the cells was studied, and their enzymatic properties were examined by immunocytochemistry. PC12 cells remained healthy within the capsules. Visible cells were found adjacent to the capsule wall within a radius of 100 to 150 μm. Mitotic figures in some of the enclosed PC12 cells were taken as evidence for continued cell growth within the capsules. Mitosis was observed up to six months following capsule placement in the brain. Approximately ten layers of PC12 cells located adjacent to the capsule wall were TH positive in correspondence with the region of viable cells. We conclude that the procedure of encapsulation allows long-term implantation of TCL into the brain.

403.1


Recently, rats with nucleus basalis magnocellularis (NBM) lesions 14 mo. previously, were reported to display neuropsychological deficits similar to those seen in human Alzheimer's brains (Arendash et al., Science 236:902,'87). We report that, using a similar excisional lesion paradigm, we did not find similar neuropathology, although the rats were impaired on biochemical, behavioral, and electrophysiological indices. Six F-344 male rats of the NBM. Three weeks post-surgery, lesioned and unlesioned controls (n=6) were tested in a water maze task (10 trial blocks, 1 block/day, 4 trials/day). Fourteen mo. later, 4 rats from each group were randomly assigned to 3 trial blocks each. Immediately after behavioral testing, these 8 rats were implanted with cortical electrodes for freely moving EEG recording. The rats were then sacrificed for histological examination with acetylcholinesterase (AChE) histochemistry, cresyl violet, and Bielchovsky silver staining. The remaining 4 rats were sacrificed for measurement of cortical choline acetyltransferase (ChAT) activity.

The NBM lesioned rats had 22.38 depilation of cortical ChAT 14 mo. after lesioning relative to controls. The lesioned rats were impaired relative to controls on acquisition of the maze task but did reach equivalent levels of performance during trial blocks 6 to 10. Fourteen mo. later, lesioned rats were impaired relative to controls on retention and reacquisition of the maze task. Although the biochemical and behavioral deficits displayed by the lesioned rats were relatively mild, EEG recording revealed high amplitude waves which were absent in control rats. While AChE positive neurons in the NBM were reduced in the lesioned rats' brains, silver staining revealed no pathology resembling cortical plaques.

403.2

INCREASED EXTRA-ADRENAL CHROMAFIN CELLS IN ADULT FISCHER-344 RATS: AN IMMUNOCYTOCHEMICAL EVALUATION. O. Yang*, R.F. Metzuma and R.I. Rapport. Lab. of Neurosci, NIA, NIH, Bethesda, MD 20892

The number of extra-adrenal chromaffin cells in male Fischer-344 rats is strikingly increased with age (Partanen et al., Neurobiology of Aging 5:105-110, 1984). In this study, the mechanism of the senescent increase was addressed using immunocytochemical methods. A monoclonal antibody against the 5-bromo-2'-deoxyuridine antibody, indicating that the age-related increase is not due to cell proliferation. To examine if a glucocorticoid receptor (GR) mechanism is involved in the senescent increase, the temporal pattern of GR immunoreactivity in the extra-adrenal chromaffin cells was followed and was compared with that in adrenal chromaffin cells. No detectable changes in immunoreactivity were found in extra-adrenal chromaffin cells, whereas the immunoreactivity decreased with age in adrenal chromaffin cells. The persistence of GR in extra-adrenal chromaffin cells and the correlation of GR immunoreactivity with distinct aging rates of the chromaffin cells suggest that the GR is involved in the numerical increase of extra-adrenal chromaffin cells in the aging rate.

THE AGING PROCESS I
30.5 Spatial Learning Deficits in the Aged Male Rat: Neuroanatomical Correlates

30.4 The effect of estrogen-induced acyclicity on estradiol receptor density in the brain. Mice (4.5 mo, n=4/group) were injected sc with oil vehicle or estradiol killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then optical density (OD) was evaluated using the ImageMeasure software system.

30.4.5 Global fibrillar acidic protein mRNA increases with age in mouse cortex.

30.4.4 Effect of lipid peroxidation on glucose transport in astrocytes: Kinetic data for pyruvate, T-Couj', G-M lithium', FF Ahmed', D.I. Cowen' and AY Sun'. Institute of Neuroscience, National Yang-Ming Medical College, Taipei, Taiwan, ROC, and Departments of Biochemistry and Genetics, University of Missouri, Columbia, MO 65203.


30.3.8 The effect of estrogen-induced acyclicity on estradiol receptor density in the brain. Mice (4.5 mo, n=4/group) were injected sc with oil vehicle or estradiol killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then optical density (OD) was evaluated using the ImageMeasure software system.

30.3.7 The evidence of estrogen-induced acyclicity on estradiol receptor density in the brain. Mice (4.5 mo, n=4/group) were injected sc with oil vehicle or estradiol killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then optical density (OD) was evaluated using the ImageMeasure software system.

30.3.6 Aging-related changes in adrenal tyrosine hydroxylase

30.3.5 Spatial Learning Deficits in the Aged Male Rat: Neuroanatomical Correlates


30.3.3 Dietary restriction of total calories increases both the median and maximum lifespan span of rodents and attenuates various age-related biochemical changes. We hypothesized that dietary restriction may affect the rate of aging by changing gene expression. Adrenal medullary catecholamine metabolism increases markedly during aging. Therefore, we measured enzymes of dopamine (DA) and acetylcholine (ACH) and associated acetylcholinesterase (AChE) activity increased 2-3 fold from 2 to 32 months of age. These changes in TH were paralleled by increases in dopamine (DA) content. There were no changes in the DH enzyme. Contrary to expectations, lifelong dietary restriction failed to attenuate the effect of aging on TH in fact, food restriction decreased adrenal tissue content of catecholamines and TH. Preliminary results show age-related increases in adrenal TH mRNA. We are currently evaluating dietary restriction on TH mRNA.

30.3.2 The evidence of estrogen-induced acyclicity on estradiol receptor density in the brain. Mice (4.5 mo, n=4/group) were injected sc with oil vehicle or estradiol killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then optical density (OD) was evaluated using the ImageMeasure software system.

30.3.1 The evidence of estrogen-induced acyclicity on estradiol receptor density in the brain. Mice (4.5 mo, n=4/group) were injected sc with oil vehicle or estradiol killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then optical density (OD) was evaluated using the ImageMeasure software system.
403.11
LEU-ENKEPHALIN (L·NK) INHIBITS IN VITRO K+ -STIMULATED ENDOGENOUS DOPAMINE RELEASE FROM THE CALYCEAL MOLLUSCUM OF YOUNG VS. OLD MALE RATS.

CX were removed from young (2-month) and old (20-25month) rats and placed in small superfusion chambers containing either buffer or buffer containing 10-8M or 10-6M ENK and superfused at a flow rate of 10ul/min. After 30 minutes equilibration effluent samples were collected every 10 minutes. During sampling 5× the media were replaced with similar media containing 100% K+. Samples were analyzed using DA using HPLC-ED. The area under the K+ stimulated release curve for each group were analyzed for significant differences. In young rats ENK reduced K+ evoked DA release (10-6M = 20±8%, 10-5M = 38±10% vs. control 76±5%). As shown previously, under control conditions old rats release significantly less DA than young rats. However, ENK evoked DA release in old-AL decreased significantly more than in young-AL. Young aged rats ENK had no effect on DA release regardless of the dose used (10-6M = 5±10%, 10-5M = 27±10%). These data suggest an important differential role of opiates in the control of DA release from the CN among young and aged animals.

403.13
Subtractive Cloning Used to Investigate Age-Related Changes in Gene Expression in Rat Brain. M.J. Blake, J. Farnam, A. Fortune* and J.U. Holbrooke. Lab. Molecular Genetics NIA (GR), Baltimore, MD. 21224 and Radiation Oncology Branch, NCI, Bethesda, MD.

The brain provides an advantageous model to assess the contribution of the genome to the aging process due to its large diversity of gene products and several well characterized, age-related structural and physiological alterations. We have used subtractive hybridization and differential expression in RNA expression that occur with aging. Subtraction hybridization cloning was used to generate a cDNA library enriched for genes that are differentially expressed in aging rat brains. 35S-EDNA from either young adult (6 month) or adult (24 month) rats were used to generate a differential expression library. The results indicate that changes in expression with age show high individual variation and may be localized within specific brain regions. (Support: MacArthur Foundation; NRC Res. Assosci. to NIB)

403.14
REPEATED ADMINISTRATION OF α-MSH DOES NOT ALTER THE INCREASED ANTIPHYTIC EFFECT OF α-MSH IN AGED RABBITS. L. E. Dill*t, P. J. Martin* and T. H. Lipton* (SOF: R. E. Dill). Dept. of Physiol., The Univ. Tex. Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.

α-MSH, an endogenous neuropeptide, is potent antiphytotic when administered centrally or peripherally. The concentration of α-MSH in the brain decreases with age, and older rabbits exhibit greater sensitivity of the gut to the phytogenic peptide when tested against the central reaction to α-MSH in aged rabbits via repeated injections. Rabbits were assigned to one of four groups: old female (5-6 years), young female (2 years), old male (5-6 years) or young male (2 years). The rabbits were male beagle by L. V. I. Cross and were given 500 and 500 mg of α-MSH. After 2 days, the rabbits were tested again for antiphytotic response to α-MSH. Neither aged nor young rabbits showed altered responses to the peptide after prolonged treatment. These results indicate that the hypersensitivity of aged animals to α-MSH cannot be reduced to the level seen in younger animals, and that there is no development of tolerance to repeated injections of α-MSH.

Supported by NIH Grant AG00109 and NINCDS HS10046.
403.15
(SPO: H. Levitan). Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.
To determine the functional significance of reported losses of brain serotonin receptors with age, we measured changes in local cerebral glucose utilization (LCGU) and behavioral responses to drug treatments in young and aged rats at 15, 15 min, and 60 min after MCPP. The highest LCGU was observed in the striatum and ataxia in aged rats was greater than in young rats. The LCGU data were consistent with the findings of other studies. The behavioral responses to MCPP, a preferential serotonin-1b receptor agonist, were measured with the quantotive, autoregulatory, and phosphocytosis method in groups of 6-8 male Fischer rats, aged 3, 12, or 24 mo, at 0, 15, and 90 min after MCPP. All MCPP concentrations were tested for 2 h after MCPP injection in LH in each age group. The LCGU response to MCPP was determined by HPLC analysis in 3, 12, and 24 mo rats.

403.16
MODIFICATION OF PHORBOL ESTER BINDING AND PKC ACTIVITY IN VARIOUS BRAIN AREAS OF AGED RATS. F. Battaini, R. Del Vescovo*, S. Goveoni*, C. Lopez*, M. Trabucchi. Chair of Toxicology, II University of Florence, Scuola Normale Superiore, Univ. of Milan, +Dept. of Pharmacology, Univ. of Bari, ITALY.
[3H]Phorbol 12,13-dibutyrate ([3H]-PDBu) binding was investigated in various brain areas of young and aged male rats. No age-related modification in Kd of [3H]-PDBu binding was observed in cortex, hippocampus and cerebellum. In hypothalamus Bmax was decreased by 39% whereas in pituitary Bmax was increased in old rats. PKC activity, measured after partial purification of the enzyme, using histone III as substrate, was reduced in soluble fractions prepared from the cortex (61% decrease) which does not display changes in [3H]-PDBu binding. The discrepancy between these results of phosphorolyzing activity data could be related to an age-related modification in enzyme sensitivity to the physiological activators not involving the phorbol ester binding site. The reduced availability of PKC in soluble fraction may affect those neuronal mechanisms not involving a transfer (and therefore the activation) of PKC molecules from the cytoplasmic pool to membranes.

403.17
The effects of radiation and aging on motor function are similar (e.g., axiaca, loss of balance and coordination), and studies have revealed that changes that take place in nigrostriatal dopamine (DA) cells lead to degenerative motor disorders. A proposed mechanism for the changes produced by aging is the generation of and inability to scavenge free radicals. Because ionizing radiation generates free radicals, the purpose of this study was to investigate the functioning of nigral DA cells in aged animals. To accomplish this, we assessed the behavioral response of DA neurons in an attempt to determine a common mechanism underlying motor deficits observed with radiation and aging.

403.18
Pathlength analysis of age-related dendritic regression. R. Pentney, H. H. Ng., Dept. of Pharmacology, Univ. of Calif. at San Diego, La Jolla, CA 92037.
A new method for analysis of dendritic regression in the Pankin cell (PC) network showed that age-related PC regression was confined to terminal dendritic limbs (TL) and that it progressed by gradual shortening of TL rather than by deletion of TL at junctions (Pentney et al., Anat. Rec. 220:75A, 1988). Subsequent application of the method to PC networks from alcoholic rats (Pentney and Queackenbush, Alcoholism Clin. and Exp. Res. 12:324, 1988) suggested a need for a computerized test for age-related regression. The present study was designed to test this need. The PC dendritic regression risk score was developed and applied here to networks from aging rats, which will be applied subsequently to networks from aging, alcoholic, and cerebellar cortices. Cerebellar cortices from Fischer 344 rats, 10, 18, and 28 months of age, were prepared by the Golgi-Cox procedure. Randomly selected PC networks from coded parasegittal sections were drawn and then measured on a digitizing tablet. Pathlengths for all terminals were obtained by computer analysis of these measurements, then averaged and sorted for minimum and maximum values. The data revealed that the maximum dendritic pathlength increased significantly in PC networks of 18 month old rats (p<.05). Ongoing analysis of frequency histograms of pathlengths will provide data reflecting changes in density of terminals within networks. (NIAAA grant AA 05592)

404.1
SOUND PRESSURE LEVEL AND ADRENERGIC INFLUENCES ON AUDIODENIC SEIZURES IN DBA AND PRIMED C57BL MICE. C. E. Lints, A. Naya ile*, and D. Capruso*. Dept. of Psychology, Northern Ill. Univ., DeKalb, IL 60115.
Experiment 1 examined the relationship between sound intensity and audiogenic seizures (AOS) activity (Leyden, 1978). Twenty-one-day-old and 30-day-old genetically seizure prone DBA mice. At both ages, and for the intensities tested, AOS activity increased as sound intensity was increased above seizure threshold. However, the higher intensities were less effective in eliciting the full AOS syndrome in the older mice. In Experiment 1, antagonistic prazosin produced anticonvulsant effects in 21-day-old DBA mice. The alpha-2 antagonist yohimbine failed to exert proconvulsant effects, however, although the higher doses produced anticonvulsant effects. In Experiments 3 and 4 genetically seizure resistant C57BL mice were first rendered AOS sensitive by acoustic priming at 1, 18, days-of-age and then tested as in Experiments 1 and 2 when they were 21 days old, replicating the results with DBA mice. In Experiment 3, the beta-1 antagonist atenolol produced anticonvulsant effects in the primed mice (Experiment 4). The results suggest that primed AOS in C57BL mice may represent a neurohumoral imbalance or phencyclidine of the DA scheme, and that the midbrain adrenergic hypothesis may extend to primed C57BL mice.

404.2
FORKER BRAIN NORNEPHRINES (NE) LESIONS DO NOT ELIMINATE THE EFFECTS OF ENRICHED ENVIRONMENTS ON SPATIAL MAZE PERFORMANCE. M. C. Manc, Susan J.E. Wurtha, Bruce A. Pappas, and Shanker Ramak*, Dept. of Psychology, Carleton Univ., Ottawa, Ont., K1S 5G4.
Previous research has shown that neonatal systemic 6-hydroxydopamine (6-OHDA) lesions of all areas, enriched (E) vs impoverished (I) rearing. Since this treatment not only lesions forebrain NE terminals but also causes a peripheral syndrome, either of these effects or their combination could alter the response to E/I rearing. Accordingly, we examined the effects on only the brain by intraventricular 6-OHDA in the neonate or 6-OHDA lesion of the dorsal NE bundle in the adult. At 24 and 48 hours after injection, rats received bilateral intraventricular injections of 6-OHDA, 4 μg, administered either to control dopamine terminals or to specific left neostriatal NE terminals. The rats were raised in I or E environments respectively for 35 days. The multilevied rats raised E-OHDA (4 μg) into the dorsal tegmental bundle and were subsequently housed in E or I conditions for 42 days. Following this treatment the rats were extensively tested in a modified Webb-Wiliams maze. Both the infant and the adult E-controlled rats were superior to their impoverished counterparts. Furthermore, although both the neonatal and the adult lesioned rats showed substantial and selective losses of forebrain NE terminals, these rats also showed a performance enhancing effect of E rearing. We conclude that lesioning of forebrain NE by itself does not eliminate the beneficial effects of chronic exposure to an E environment on spatial problem solving.

In the social interaction (SI) test, anxiety is generated by the novel situation of placing unfamiliar pairs of rats together in a familiar setting. While in the elevated plus maze, exposure to an elevated open alley results in an approach-avoidance conflict in rats. In the present study, SI, the classical anxiety response, is induced by serotonin agonists, 5-Me-ODT, and 8-OH-DPAT. Increases in SI were blocked by antagonists of the 5-HT receptor, such as the non-benzodiazepine anxiolytic, buspirone. This suggests that anxiety is mediated by serotonin and excitatory amino acid systems.

In this animal model, non-benzodiazepine anxiolytics (5-HT1A ligands) are thus reliably detected. At present, we view these substances that only inhibit the pup reactions at the cold plate as potential 5-HT1A ligands.
404.10

SENSITIVITY TO THE BEHAVIORAL EFFECTS OF THE TRH ANALOG MK-771 FOLLOWING TREATMENT WITH 3-ACETYLPYRIDINE (3-AP). M.S. Kreider, S. Wieland and I. Lucki (SPON: J. Winkler). Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104. Systematic administration of 3-AP to rats produces lesions of the inferior olivary complex resulting in dystonic symptoms. We studied the functional and morphological integrity of the TRH system in rats treated with 3-AP by examining the following: head shake response produced by the TRH agonist MK-771, TRH levels in 12 brain regions, and the density of TRH receptors using receptor autoradiography. Treatment with 3-AP significantly reduced the ability of MK-771 (0.63-10.0 mg/kg) to produce the head shake response. The ED50 value for MK-771 was shifted to the right and the peak response was reduced by 50% after 3-AP. TRH levels were unchanged in all brain regions following 3-AP treatment. TRH receptor density of measured using [3H]-TRH, was significantly reduced in laminae 4-10 of the cervical spinal cord. The sensibility found to the behavioral effects of MK-771 following 3-AP treatment may be associated with a down-regulation of TRH receptors.

This research has been supported by the Dystonia Medical Research Foundation and USPHS Grant GM 34781.

HUMAN BEHAVIORAL NEUROBIOLOGY III

405.2

NEUROMAGNETIC VISUAL EVOKED RESPONSES TO SINUSOIDAL GRATINGST. J. George*, C. J. Aime, and E. R. Flynn, (SPON: D. L. Arthur). Neuromagnetism Laboratory, Los Alamos National Laboratory, MS M882, Los Alamos, NM 87545. Neuramagnetic evoked responses were recorded for visual stimuli presented in the central visual field (CVF) or 8° in the right visual field (RVF). Stimuli were high contrast, intensity-modulated vertical sinusoidal gratings of 1 (Lo) or 5 (Hi) cycles per degree, that occupied 2.0° x 1.59°. Neuramagnetic data were collected at 42 sensor locations over occipital and parietal regions. Field amplitudes were sampled at 10 ms intervals from continuous waveform data to produce a temporal series of neuramagnetic field maps which were then fit by a current dipole model using least squares procedures. In this presentation we focus on data from three human subjects. Calculated RVF response sources were deeper than those for corresponding CVF stimuli. Field amplitudes and calculated current moment for the initial response peak were smaller for RVF than for CVF stimuli and generally peaked earlier. Major differences in field distribution and calculated source location and orientation were observed as a function of visual field of stimulation. Smaller but apparently significant differences were observed in the field of spatial frequency in early response components for a particular stimulus location. Low spatial frequency (SF) gratings produced more anterior and larger current moments than Hi SF for RVF stimuli.

Recently it has been suggested that patterns of hemispheric specialization vary with gender. For example, studies of aphasic and apractic patients indicate that men and women differ in terms of anterior to posterior organization of function with women demonstrating more left hemisphere (Kimura, 1983, 1987, Can. J. Psych.). In the present study, gender differences in the neurophysiological substrates underlying memory were investigated in normal subjects (10 males, 8 women) by recording the event related potentials (ERPs) from 19 scalp electrodes during performance on visual recognition tasks for recurrent verbal items (RV) and nonverbal figures (RF). The mean reaction times did not differ for the tasks, whereas the latencies of the memory-related components (N4 and P3) were significantly longer for RV and shorter for RF. Significant gender differences were also found in the intensity of activity in the left hemisphere interactions in the ERPs due to larger ERPs anteriorly for males for both tasks, but slightly larger ERPs posteriorly for females in the RV task. This was found only for the cognitive components (P2, N4, P3). The differences found in normal subjects between the sexes extend the evidence from neuropsychological studies in patient populations that men and women differ in terms of anterior to posterior organization of function.

405.4

EEG COHERENCE AND SEMANTIC PROCESSING UNDER SUBLIMINAL AND SUPRALIMINAL TASK CONDITIONS. W.D. Shipstead 11 and R.W. Boyer*. Dept. of Biological and Physiological Sciences, Maharishi International University, Fairfield, IA 52556.

Twenty-four subjects were presented 240 randomized trials of a Lexical Decision Task (LDT) while connected to a 16 electrode EEG montage. EEG data were recorded in pairs for each of three conditions: eyes-open, eyes-closed, and a target letter string which remained on the monitor until the subject pressed one of two buttons, word or nonword. Following the lexical decision the subject gave a verbal report identifying the word as word or nonword. The performance of the subject was associated with more efficient processing in fast prime trials but less efficient processing in slow prime trials. Coherence in left temporal-parietal areas showed the opposite trend but only in related prime-target trials. The relationship between high interhemispheric coherence and semantic priming was significantly different in fast vs slow prime trials but only in the unrelated prime-target trials. A model relating flow of information during cognitive processing and coherence is discussed.

405.5


The principal component of the word auditory evoked potential (EP) is the N1, a negative peak at 100 msec. We examined N1 amplitude in waking and stage II-IV sleep, and compared it to the most prominent sleep EP, the N2 (latency 200 msec). N1 amplitude of 28% of stimuli were 1.00Hz tones (1.0 sec ISI, 60dBHL). Response to stimulus deviance was evaluated by randomly presenting the deviant stimuli, a different complex novel sounds, each occurring in 10% of trials. The N1 latency was maximal at amplitude at frontal scalp sites, with minimal amplitude at posterior scalp sites. Deviant stimuli generated a mismatch negativity (MMN) of 4.0 uV at frontal sites. During stage II sleep N1 amplitude was reduced. MMN was present but reduced from 1.0 set to 1.0 uV. In stage III-IV sleep N1 was abolished at all sites. AEPs during stage IV-IV sleep were dominated by a complex novel sound (latency 252 msec). N2 amplitude increased in response to deviant stimuli in all sleep stages. This study suggests that the intracranial sources of AEPs change from wakefulness to sleep. Furthermore, the enhanced response to deviant sounds observed during waking and all sleep stages indicates the differential processing of auditory stimuli persists during sleep.

405.6


In this selective attention task, targets were rare longer (170ms) tones of a designated pitch, imbedded in a sequence of 100ms standard tones. An additional simple reaction time (SRT) condition required a response to an unvarying standard. Event related potentials (ERPs) were recorded for 14 sites. In this SRT condition both negative (N400) and positive (P3) peaks were similar for both groups. Selective attention effects were evident in the ERPs to standards. Attended standards ERPs (SRT) were characterized by a 1000Hz (N1) and 2000Hz (P2) difference wave (attend-unattend standards) revealed a statistical significant effect for N1 (late N1) and P2 (late P2 only) components. No significant differences were found between the tasks or sexes in the reaction time or error rates. These sex differences may be due to the interactions in the ERPs due to larger ERPs anteriorly for females for both tasks, but slightly larger ERPs posteriorly for males in the RV task. This was found only for the cognitive components (P2, N4, P3). The differences found in normal subjects between the sexes extend the evidence from neuropsychological studies in patient populations that men and women differ in terms of anterior to posterior organization of function.

405.7

EVENT-RELATED POTENTIALS TO VISUAL "POP-OUT" STIMULI. L. Lucke & S.A. Hillyard (SPNS: O. Manjung). Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92033.

When a visual display contains an item that differs from the background on the basis of a basic visual feature, that item can be discriminated between the early, pre-attentive stage of processing. In subjective terms, it appears to "pop out" from the rest of the display. We recorded event related brain potentials from 10 young adults while they engaged in a task that required them to discriminate pop-out stimuli. Participants were presented with visual displays of 8 randomly located colored bars. On no-pop-out trials (p=5), all 8 bars were blue and vertical, and on pop-out trials, half of the bars was either horizontal (p=17) green (p=17), or wide (p=17). For each run, 1 of the 3 pop-outs was the target, while the other 2 were pop-out and no-pop-out distracters. Target stimuli elicited enhanced frontal F2, posterior N2, and broad P3 components. The posterior N2 was largest at scalp sites contralateral to the side of the pop-out and may represent the operation of focal attention. Both target and non-target pop-outs elicited a frontal N2 component, suggesting an automatic orienting to discrepant items regardless of their relevance.
405.9 BRAIN POTENTIALS PREDICTIVE OF LATER PERFORMANCE ON TESTS OF REAL RECOGNITION. A.C. Faller, J. C. Wood, and G. McCarthy. Neuropsychology Laboratory, VA Hospital (16B1), West Haven, CT 06511 and Dept. of Neurology and Psychology, Yale Univ.

Despite the connection established between the brain areas damaged in amnesia and declarative memory, the functional roles of these brain areas are currently unclear. Information derived from the electrical activity generated in these brain areas during memory processing may be useful for implicating the brain mechanisms mediating declarative memory. Event-related potentials (ERPs) may be sensitive to task demands and may provide information from human subjects engaged in complex verbal tasks. Correlations between ERPs and later memory performance have been reported previously. In these studies, ERPs elicited by words that were later remembered were more positive than ERPs elicited by words that were later forgotten.

The present experiment investigated electrophysiological correlates of memory performance in relation to retention interval and type of memory task (free recall, yes-no recognition, and/or completion priming). In the priming test (a test of nondeclarative memory), 3-letter stems corresponding to some of the 200 words presented earlier were mixed with an equal number of additional stems. Subjects were instructed to complete each stem verbally with the first word that came to mind. The results of the completion priming test were consistent with priming performance.

Scores were much greater with the short delay (45%) than with the long delay (18%), and in both cases were significantly greater than chance (10%). ERPs elicited by recalled words were relatively more positive than ERPs to unrecalled words, especially 600 ms after word onset. Similar though less consistent results were evident at both central and temporal sites and that these differences were task dependent and influenced by instruction.

Using a simple and a complex S-R compatibility task, subjects were tested on a visuomotor reaction time task with an equal number of simple and complex trials. At all four sites, the P300 declined in amplitude as a function of delay and averaged 57% (chance = 14%). Priming and priming tests were given either after a 1-min delay or after a 15-min delay.

In the present experiment, we assessed the distribution and electrical characteristics of ERPs elicited during a variable demand spatial rotation task in which subjects were asked to decide whether a spatial schema was identical to an earlier template. Task difficulty was increased by incrementing both the number of bars in the histogram and the degree of rotation. Nine subjects were fitted with ERPs, and 50 contacts were used to record the ERPs. In the complex task, compatible responses were made to upper- and lower-case words while the lower-case words required pressing the opposite button. In the simple task, compatible responses were made to upper- and lower-case words while the upper-case words were made to lower-case words required incompatible responses. As expected, RTs were influenced by task, compatibility, and instruction. The difference in the amplitude of the ERPs recorded from the occipital (O1) and frontal (F3) sites was in response to validly and invalidly cued targets appearing 14 degrees to the right or left of a central fixation point. The interval between the cue and the target varied from 200 to 600 msec. The results showed that attention can be switched within 500 msec. The amplitude of Ti to cue targets was modulated by the validity of the cue targets, particularly at parietal and central sites. Amplitude of P3 component reflected differences in probability of the valid and invalid conditions. Reaction times also revealed the costs and benefits of shifting attention to either the invalid or valid hemifield.

These results corroborate previous findings of parietal involvement in the directing of spatial attention.

405.10 VISUAL SELECTIVE ATTENTION TO LINGUISTIC STIMULI: INTRACRANIAL ERP STUDIES. A.C. Faller, J. C. Wood, and G. McCarthy. Neuropsychology Laboratory, VA Medical Center, West Haven, CT 06511 and Dep. of Neurology and Psychology, Yale Univ.

We have previously demonstrated that the ERPs associated with the processing of concurrent visual and auditory stimuli (Nobre & McCarthy, Soc. Neurosci. Abstr. 1987). The spatial-temporal characteristics of the ERPs were recorded in multiple patients with neurological disorders, and it is generated by multiple neural sources. Since neural generators cannot be determined from scalp distributions, scalp ERPs were obtained from a group of patients being evaluated as candidates for surgery to re- tain medically intractable seizures. Each patient had chronically implanted unilateral or bilateral posterior-temporal (PT) multicontact depth electrodes. The first several contacts of the PT probe(s) sampled activity in extrastriate visual cortex, while the deeper contacts were in the medial temporal lobe.

Words comprising two stories were randomly intermixed and displayed briefly and successively on the center of a computer display, with words in each story displayed in a single color (red or green). Subjects were instructed to read silently either the red or green story. Pretreatment of the attended story was tested immediately at the end of each run.

Like the ERPs recorded from the scalp, the intracranial ERPs recorded from the PT probe(s) superficial contacts showed a large attentional effect in four out of five patients. The morphology and time-course of the ERPs were strikingly similar to that recorded from the occipital (O1) scalp electrode in normal subjects. The intracranial effect, however, was approximately an order of magnitude larger in amplitude and opposite in polarity to what was observed in its scalp counterpart. This polarity inversion suggests that one generator of the selective attention effect recorded at the scalp may be located in the extrastriate cortical region.
405.15  NEUROPSYCHOLOGICAL AND ELECTROPHYSIOLOGICAL DIFFERENCES IN TURNER SYNDROME, J. Glueckert1, M. Schachtner1, D. Shucard1,2, Depts. of Neurology, Pediatrics, and Psychiatry, SUNY at Buffalo School of Medicine, Buffalo NY 14203.

In order to further clarify the cognitive phenotype in Turner Syndrome (TS), electrophysiological and behavioral measures were obtained in TS girls between the ages of 8 and 14 yrs. Subjects were evaluated with subtests of the Halstead-Reitan Battery to quantify spatial and sequential processing skills. Electrophysiological measures were obtained using auditory cortical evoked potentials (EP) to probe differences in electrical activity between homologous areas of the brain during the performance of visual-spatial tasks.

Neuropsychological findings indicated that although there was a heterogeneous pattern of test scores among subjects, they uniformly showed poor performance on the localization component of the Tactual Performance Test. Electrophysiological findings showed that the patterns EP asymmetry obtained during visual task performance appeared to be related to neurocognitive measures. These data indicate that neurocognitive deficits are present in TS but may be more complex than a general disturbance in spatial processing. Further, neurocognitive deficits may be related to electrophysiological indices of brain organization. Supported in part by Hoffman-LaRoche, Inc.

405.16  FREQUENCY DEPENDENCY OF MIDDLE LATENCY AUDITORY RESPONSES OF HUMANS. M.L. Collot1, R.A. Johnson and J. Brain, Department of Psychology, UCLA, Los Angeles, CA 90024.

The P1 component of middle latency auditory event related responses is modulated by state of arousal and by stimulus repetition rate in both humans and cats. The neural generator of P1 is a matter of controversy, having been hypothesized to lie either within the rostral reticular activating system or in the primary auditory cortex.

P1 is a difficult component to analyze since its amplitude is relatively small and is often contaminated by muscular artifact. For these reasons, not all subjects demonstrate reliable P1 components. We attempted to determine whether the neural tissue generating the human auditory P1 (occurring at latencies of 50-80 msec) is frequency dependent. Frequency dependency would be suggestive of a cortical rather than reticular source. P1s were measured to 800 Hz tone bursts, 100 msec in duration, presented at either 2 sec or 0.5 sec interstimulus intervals (ISIs). It has previously been reported that P1 amplitude is attenuated at shorter ISIs. In the critical third condition, 800 Hz tone bursts were presented at 2 sec ISIs, with three 1000 Hz tone bursts intervening. Thus, in this condition, the frequency dependent ISI was 2 sec, while the frequency independent ISI was 0.5 sec.

Recordings were made on 11 subjects (bandpass filter settings of 10-300 Hz, Cz to linked ear reference, averages of 250 trials). P1 was measured relative to N1, the preceding negative, identifiable and consistent P1s were observed in 5 of these individuals. The results suggest that the neural generators of the human P1 are frequency dependent; the amplitude of the P1 elicited by the 800 Hz tone in the mixed sequence was similar to that obtained by the 800 Hz presented at 2 sec ISIs. These results are suggestive of a cortical generator for P1. Investigations with additional subjects are being conducted. (Supported by NIMH 37450-07)

405.17  STIMULUS INTENSITY EFFECTS ON MONKEY EVENT-RELATED POTENTIALS (ERPs): PARALLELS TO HUMAN COGNITIVE-DECISION-MAKING, G.S. Holmes1, J. A. Friston1, D. Swick1,2, Departments of Psychiatry and Neuroscience, UIUC, and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Stimulus intensity effects on ERPs, as characterized by augmenting-reducing (AR) waves, have been proposed to index a central mechanism for gating memory processing and have been correlated with levels of biogenic amines. In order to study these effects, AR characteristics in auditory ERPs were recorded in squirrel monkeys (Saimiri sciureus) from chronically implanted epidural electrodes. The relationship between stimulus intensity and EP component amplitude was examined at frontal midline, vertex, and temporal sites. Augmenting was defined as a monophasic increase in peak-to-peak amplitude for the first two major peaks, across three of the four intensities presented (50, 60, 70, and 80 dB SPL). Additionally, the response to the highest intensity was required to have the greatest amplitude. Any other result was defined as reducing, with differences in the AR profile in spatial processing. Frontal sites most commonly showed an augmenting profile, while temporal sites often showed a reducing profile. Despite amplitude variations between sessions, AR profiles were found to be stable over months for individual subjects. A Spearman rank-order correlation coefficient based on all subjects' data indicated a statistically significant correlation between amplitude/intensity slopes at temporal sites and P300 component areas elicited at parietal sites using an auditory oddball paradigm (r = 0.80, p < 0.005). These results indicate parallel findings in human and monkey AR profiles with regard to AR profile differences between sites and P300-AR relationships.

405.18  EFFECTS OF CLONIDINE ON P300-LIKE POTENTIALS IN SQUIRREL MONKEYS. D. Swick1,2, J. A. Friston1, D.C. Asaad, J. A. Pineado2, T. C. Holmes3, Dept. of Neurology and Psychiatry, UIC, Chicago, IL 60612 and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Previous experiments in this laboratory have shown that bilateral lesions of the noradrenergic nucleus locus coeruleus (LC) decrease the amplitude of the monkey P300. To further assess the role of LC in modulating the orienting and attentional processes indexed by the P300, we systemically administered the alpha-2-adrenergic agonist clonidine to squirrel monkeys (Saimiri sciureus) and recorded their event-related potentials (ERPs) from chronically implanted epidural electrodes. Two animals received extensive training in a 90-10 auditory oddball paradigm. Their P300 areas remained relatively stable throughout six pre-drug sessions and showed no habituation. Three untrained monkeys were presented with tones in a passive 90-10-10 oddball paradigm. In separate sessions, all animals received injections of saline or of clonidine (0.05, 0.075, and 0.1 mg/kg i.p., thought to suppress LC firing) and their ERPs were recorded. At lateral parietal sites, where the monkey P300 is most pronounced, three of five subjects (2 active, 1 passive) showed a dose-related decrease in P300 area and exhibited recovery to control levels in post-drug sessions. The remaining passive subjects failed to show clear P300s in both pre- and post-drug sessions, and hence their clinical data were difficult to interpret. These preliminary results support the hypothesis that the LC noradrenergic system participates in P300 production.


In a continuously presented stimulus series in which the subject is instructed to detect one of two stimuli, the amplitude of the P300 component of the human scalp-evoked potential reflects expectancy of the stimulus as predicted by the immediately preceding stimulus. The hippocampal formation has been suggested to be the site of generation of the sequential dependency of P300. A component of the tone-evoked hippocampal averaged evoked potential (AEP) in the rat has been identified that shows a sequential dependency in a manner similar to P300. The N1 component of the AEP can be described as an updatable buffer which stores the sequence of five preceding stimuli, but not the current stimulus (Deadwyler et al., Behav. Neural Biol., 44, 1985). N1 amplitude is maximal when preceded by a series of unrewarded stimuli, and is maximal when preceded by reward and (non)target trials, irrespective of the current (evoking) stimulus. In contrast, P300 amplitude is maximal when the current stimulus is different from the preceding sequence of stimuli and does not depend upon whether the stimulus is a target or non-target.

Recent studies from this laboratory suggest that P300 cannot be generated in the hippocampus, but that P300 sequence processing is represented by hippocampal sensory-evoked potentials (Hampson and Deadwyler, Behav. Brain Res., 186). This relationship provides the basis for a hypothesis that N1 serves as a critical neurophysiological substrates for cognitive functions represented by P300. The proposed interaction between N1 and P300 will be examined using a neural modeling approach. A neural network model of a serial memory acted on in N1 amplitude will be discussed. (Supported by NIDA grants DA04441, DA03502, and DA00139 to S.A.D.)
**406.1** NEURAL FATTY ACID PROFILE IN WEANED RATS IS SUSCEPTIBLE TO SHORT-TERM CHANGES IN DIETARY FAT COMPOSITION. J. A. K. DEVER and C. E. GREWAL (SPON: G. H. ANDERSON). DEPT. OF NUTRITION, FAC. MED., UNIV. OF TORONTO, TORONTO, ONT., CANADA. N5S 1A8.

We have shown that dietary fatty acid (FA) composition, in the absence of essential fatty acid (EFA) deficiency, alters behavior of weaned rats, including spatial memory, pain sensitivity, and protein selection. Within 4 weeks of feeding: i.e., neither long-term feeding for several months nor EFA deficiency were necessary to observe a functional effect of dietary FA. In general, dietary changes were accompanied by altered lipid composition of brain membranes, the same 20% (w/w) soybean oil and lard diets were used for 6 weeks old male Sprague-Dawley rats for 4 weeks. Dietary fat influenced FA profiles of phospho-lipids (PL) in myelin, synaptosomes, mitochondria and microsomes; the degree of change was similar across all subcellular fractions and all PL (PC, PS, PE, CL, GL) except PI which was relatively unaffected. Polysaturated fats, except arachidonic acid (20:4ω6), were influenced by dietary fat more than saturated or monounsaturated FA.


We have shown that phosphatidylcholine, the most abundant phospholipid in cell membranes, is synthesized primarily via the GDP-choline pathway in most cell types. This route utilizes CTP and phosphocholine as its rate-limiting substrates. Thus, external factors that affect the availability of these precursors could influence phosphatidylcholine synthesis. We are investigating the effects of cytidine on cellular nucleotide content and on the incorporation of [3H]-choline into phosphatidylcholine and water-soluble precursors. Rat pheochromocytoma cells (PC-12) were incubated in serum-containing DMEM medium in the presence of [3H]-choline (15mCi, 7.4mM), with and without cytidine (100μM). After 15 and 30 minutes of incubation, incorporation of [3H]-choline into phosphatidylcholine was increased by 50 and 42%, respectively, in cytidine-supplemented cells, while labeled phosphocholine decreased by 43% and 32%. Intracellular CTP levels were doubled after 15 minutes of incubation in the presence of cytidine, and rose even higher thereafter. In similar experiments, when cells were incubated with various concentrations of cytidine, these effects were dose and time-dependent. A pulse-chase study with labeled choline also showed that most of the radioactivity initially associated with phosphocholine was gradually converted to labeled phosphatidylcholine. In the presence of cytidine, this conversion was accelerated by 36 ± 4% at all times studied. These results suggest that cytidine could be a critical regulator of phosphatidylcholine biosynthesis, possibly by elevating intracellular levels of CTP.

**406.3** MICROVESSEL MEMBRANE LIPID COMPOSITION AND AGING. W. N. WILLIAMS and T. R. MCKEIL, DEPT. OF NEUROLOGY, UNIV. OF ROCHESTER SCH. OF MED. DENT., ROCHESTER, NY 14642.

Microvessels were isolated from the cerebral cortex and cerebellum of 10, 20 and 27-30 month old mice. Membrane lipids were extracted from both microvessel and brain parenchymal fractions, and phospholipids and cholesterol separated by thin-layer chromatography (TLC). Cholesterol, phospholipid (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM) were detected on TLC plates. Phosphatidylcholine (PC) was the most abundant phospholipid in microvessel membranes. Cholesterol content was determined by the cholesterol oxidase method. The degree of change varied from 5% for palmitic (C16:0) and 10% for oleic (C18:1ω9) and 7% for linoleic (C18:2ω6) and 10% for linolenic (C18:3ω3) acids. Total cholesterol content of microvessel membranes was determined spectrophotometrically. The data suggest that, 1) microvessel membranes from 27-30 months old mice exhibit a marked increase in the level of unsaturation, attributable primarily to an apparent increase in arachidonic (20:4) and docosahexaenoic (22:6) acids, 2) cholesterol content increases in both microvessel and brain parenchymal membranes, and 3) in contrast to parenchyma, the relative content of the major phospholipid classes in microvessel membrane remains essentially unchanged over the age period studied. These findings suggest that microvessel endothelial membranes undergo compositional changes that could potentially influence microvascular production of eicosanoids, and alter blood-brain barrier function.


One of the 2 major gangliosides recognized by the mononuclear antibody JONES (Constable et al 1986 Nature 324:595) is 9-acetyl-GD3 (Blum et al 1987, JBC, 262:17615). JONES immunoreactive gangliosides have been implicated in neural cell migration as well as in neural development (Constable et al 1988, Neurosci Lett 8:540). We have used a monoclonal antibody isolate 9-acetyl-GD3 for functional studies. Our purification scheme was simplified with respect to standard procedures to minimize the number of steps required to obtain pure product. The procedure: the intact, unextracted tissue was subjected to sequential chromatography: first on ion-exchange column, then twice on silicagel (silicogel) column, all eluted with gradients of iodic acid/methanol/water 1:1 0-9-acetyl-GD3 was identified on the basis of i) HPTLC mobility and location with C11 and C19; ii) JONES immuno-reactivity. Recovery on to C18 by mild base treatment. Supported by grants R01-CA22963 and NS2601965.


The monoclonal antibody JONES (Constable et al 1986, Nature 324:595) recognizes a small family of rare gangliosides (Schlossman et al 1986, Neurosci Lett 591:5). We developed a method to isolate and characterize the gangliosides recognized by 9-acetyl-GD3 (Blum et al 1987, JBC, 262:17615). We obtained them by ion-exchange chromatography, a ganglioside fraction enriched for the second major JONES immunoreactive ganglioside, which has HPTLC mobility at 1G and G3 and G4 (the base sequence of the second major ganglioside). We isolated and characterized the ganglioside fraction and analyzed the composition and immuno-reactivity of the gangliosides. The ganglioside fraction was identified by the mAb A285, which was previously shown to be specific for G3c (Kesel & Yu 1983, Brain Res. 275:155).

We conclude that the second (the first one being 9-acetyl-GD3) major ganglioside is a base-tautomer of 9-acetyl-GD3, which we designate as 9-acetyl-GD3. Supported by grants R01-CA22963 and NS2601965.

**406.6** GANGLIOSIDE COMPOSITION OF CHOROID PLEXUS BRAIN TUMORS IN TRANSIGenic MICE. M. ElABBADI, A. MASSING, AND T. N. SEYFIED. DEP. OF BIOL., BOSTON COLL., CHESTNUT HILL, MA 02167 AND SCH. OF MED., UNIV. WASHINGTON.

The ganglioside composition of two cholesterol plexus tumors (papillomas), SV (1985) and SV-MK (79), was examined in the brain of transgenic mice. These papillomas are in vitro tumors that develop spontaneously in adult transgenic mice carrying integrated copies of XhoI rat gangliosides. The ganglioside composition of these tumors was compared to that of normal mouse brain and to a chemically induced ependymoblastoma growing subcutaneously in the flank. The total ganglioside sialic acid content (μg/100 mg dry weight) of the two papillomas was 76±2 (four SV-MK tumors pooled for a single sample) and 139±4 (the mean of two independent SV-MK tumors). These concentrations were higher than that of the ependymoblastoma, 39±6 (N=4), but markedly lower than that of the adult mouse brain (450±50). Although N-acetylgalactosaminic acid (NANA) is the only galactoside sialic acid in adult mouse brain, both papillomas and the ependymoblastoma contained significant amounts of N-glycolylneuraminic acid (NGNA). GM3-NANA and GM3-NGNA were the predominant ganglioside species in both papillomas and the ependymoblastoma. From thin-layer chromatography and, the ganglioside composition of the GM3 ganglioside appears to be more structurally homogeneous in the papillomas than in the ependymoblastoma. In conclusion, the ganglioside composition of the papillomas contained significant amounts of mouse brain gangliosides (GD1a, GD1b, GT1b, and GQ1b). These gangliosides may not be native to the tumors, but represent contaminants from normal brain tissue surrounding the tumors. (Supported by NIH grants NS23355, NS24826, and NS23374).
406.7  
GANGLIONEURON MEDIATION OF GABA DEPENDENT PROTEIN KINASE AND cAMP ACTIVATION IN THE PHOTORECEPTOR CELL. R.A. Yates, J.B. Walters*, C.L. Wood*, S.M. Stock* and J.D. Johnson*. Departments of Pathology, Pharmacology, and Physiological Chemistry, Ohio State University College of Medicine and College of Dentistry, Columbus, Ohio 43210. 

Phosphorylation of proteins in membranes isolated from sciatric nerve of normal adult rabbits was examined using an in vitro assay, PAGE and autoradiography. In the absence of Ca2+ there were 3 major phosphorylated proteins (21 kDa, 2 kDa and 51 kDa). Catalytic subunit of GTP dependent protein kinase (PKA) enhanced phosphorylation of 21,31,38,40 and 49 kDa proteins. GTP, GTPyS, GTib inhibited phosphorylation by PKA of 38,40 and 49 kDa proteins with half maximal inhibition at 13-32 µM. With type III hase a as a phosphate acceptor GTP competitively inhibited PKA with Ki=110 µM. Neutral glycolipids were much less effective and free sialic acids had no inhibitory effect. GTP stimulated the activity of purified bovine brain calmodulin-dependent cyclic nucleotide phosphodiesterase, with activation being half maximal at 0.3 µM and maximal (32-fold) at 1 µM, but had no effect on PKC. These findings indicate that gangliosides may affect the activity of PKA through two mechanisms: (a) decreasing levels of cAMP by activating phosphodiesterase; (b) direct inhibition of the catalytic subunit. Supported by NIH grant MS16155, Department of Pathology and College of Medicine.

406.8  
MODULATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE IN GLIAL CELLS BY GLOUCOSE. W.C. Ww, K. Kubota, J.J. Paterson and H. Paterson. School of Medicine, University of Oklahoma Health Sci. Cntr., Oklahoma City, OK 73190.

The amino-sugar, glucosamine, is known to decrease the expression of membrane-associated cytoplasmic tyrosine phosphatase. However, cell surface gamma-glutamyl transpeptidase (GGT) activity was stimulated by 15m glucosamine in cultured glial cells (Ca, rat astrocytoma) over a 48 hr period. The increase in enzyme activity was dependent upon the entry of glucosamine into the cell and not on the inhibition of glucose transport. GGT in glucosamine-treated cells exhibited an increased sensitivity to a variety of acceptor-substrates especially cysteine, while at the same time, enzyme affinity for wheat germ agglutinin (WGA) was markedly decreased. The enzyme also exhibited an altered Vmax. Rat liver hepatocytes (2B-RL), used as a control cell, had a similar Vmax alteration but in addition had an altered Km. The data suggest that the increase in GGT activity in glucosamine-treated cells results from the appearance on the cell surface of a functionally distinct iso-form of GGT. To date, it can be recognized by its disproportionate response to amino acid acceptors and by its decreased affinity for WGA.

(Was was supported in part by NIH Grant 500-NS-18755)

406.9  
ALTERATION OF INTRACELLULAR CHLORIDE CONCENTRATION AND ITS EFFECT ON THE EXPRESSION OF ACH RECEPTORS IN THE MUSCLE. R.D. Heathcote and W.J. Betz. Dep't of Physiology, Univ. of Colorado School of Medicine, Denver, CO 80226.

The mechanism by which chloride concentration regulates the expression of acetylcholine receptors (AChRs) is not known. Expression of extrajunctional (EJ) AChRs correlates well with the level of internal [Cl] under a variety of conditions. For example, in normal muscle both are relatively low, but when denervated EJ AChRs appear and, as [Cl] conductance falls, internal [Cl] rises owing to the continued action of ACh on the denervated muscle. Electrophysiological methods and internal [Cl] were measured with the use of tritiated ACh as a label and following the iodination of surface proteins with 125I-α-lactoperoxidase and partitioned (>70%) into the detergent-extracted membrane. AChR binding, [Cl] conductance with electrophysiological methods and internal [Cl] with Cl-sensitive microelectrodes. Denervated muscles were studied in culture system. Experimentally, AChR s were measured with tritiated ACh, [Cl] was measured with KCl with type IIA histone as a phosphate acceptor cAMP decreased by a factor of 10 in normal muscle under a variety of conditions. For example, in normal muscle both are relatively low, but when denervated EJ AChRs appear and, as [Cl] conductance falls, internal [Cl] rises owing to the continued action of ACh on the denervated muscle. Electrophysiological methods and internal [Cl] were measured with the use of tritiated ACh as a label and following the iodination of surface proteins with 125I-α-lactoperoxidase and partitioned (>70%) into the detergent-extracted membrane. AChR binding, [Cl] conductance with electrophysiological methods and internal [Cl] with Cl-sensitive microelectrodes. Denervated muscles were studied in culture system. Experimentally, AChR s were measured with tritiated ACh, [Cl] was measured with KCl with type IIA histone as a phosphate acceptor cAMP decreased by a factor of 10 in normal muscle.

406.10  
DENSITY OF 3H-STX BINDING SITES IN PREMYELINATED AXONAL MEMBRANE IN MAMMALIAN OPTIC NERVE. G.O. Waxman, J.A. Black and J.M. Richie2. Dep'ts. of Neurology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510, and V.A. Medical Center, West Haven, CT 06510.

As part of a study on membrane assembly in myelinated axons, we studied STX-binding in myelinated optic nerves (ON) of normal neonatal rat and rabbit. Since virtually all their axons become myelinated in the adult, these nerves provide a model for studying precursors of myelinated axolemma. Fusion of neonatal (0-2 d) rats and rabbits were excised, weighed, pooled and homogenized, and the protein content and specific 3H-STX-binding curve determined. The maximal specific STX-binding for neonatal rat and rabbit ON was 14 and 38 fmol/mg wet wt., respectively. Surface density of axon membrane, derived by the line-intersection method, was 9.6 µm2/µm 3 (rat) and 11.3 µm2/µm 3 (rabbit). For each species, surface density of ON gial membrane was ~10% of axon membrane surface density. These results indicate a mean density of 4-7 STX binding sites per µm 2 of axolemma in neonatal rat and rabbit ON on the basis of a uniform plasmalemmal distribution of channels. Thus, both rat and rabbit premyelinated axolemma seem to exhibit an axolemmal Na channel density considerably lower than do adult unmylelinated fibers (100-200 µm 2). Since previous studies have demonstrated that neonatal rat ON exhibits TTX-sensitive action potential electrophoresis, the present results suggest that very low channel densities are capable of supporting spike electrophoresis in premyelinated axons. (Supported in part by the NIH, NIMH and VA).

406.11  
EXPRESSION OF GP5O BY CULTURED CEREBELLAR GRANULE CELLS. T. Palade*, S.M. Nicholas*, P. Carden*, and J.K. Gurd (SPON: I.R. Brown). Dep't of Biochemistry, Univ. of Toronto, Scarborough Campus, West Hill, Ont., M1C1A4 and Dep't of Biochemistry, Royal Holloway and Bedford New College, Egham, Surrey, UK. TW20 0EX.

We have previously described the brain-specific glycoprotein, GP50 and shown that it is expressed by granule cells in primary tissue culture (Heeley et al, 1987, Br. Res., 408, 65-78). In order to further characterize GP50 we have investigated its developmental expression and molecular properties in granule cell processes. Immunocytochemical staining of 10 day old cultures, using the monoclonal antibody 2G5 GP50 was confined to the perikaryal cell surface and not within the cell. Neurites showed little or no reactivity with GP50. Immunoblotting showed that the levels of GP50 increased throughout development in cultures. In cultures (2 DIV) staining was reduced and was largely restricted to cells already present in forming aggregates. GP50 was labelled following the iodination of surface proteins with lactoperoxidase and partitioned (>70%) into the detergent phase following extraction with Triton X-114. Sodium density gradient centrifugation of Triton X-100 extracts showed that GP50 exists predominantly (80%) in a 3.6S form (calculated Mr: 40K). The results indicate that GP50 is a developmentally regulated, integral, cell surface membrane glycoprotein. (Supported by grants from NSERC and MRC to JKG).

406.12  

In mixed fetal CNS cultures, growing astroglia express a membrane-associated activity that disrupt neuron-substrate interactions leading to neuronal detachment. The present study demonstrates first, that this invasive activity of astroglia can be presented by treatment with free, extracellular cAMP (cAMP), an in vitro morphogen that induces stalk morphology in astroglia. Second, the sulfated glycosaminoglycan heparan sulfate (HS) was found to inhibit neuron detachment in a reversible manner without affecting the flat morphology of astroglia. The importance of HS in neuron-substrate interactions leading to neuronal detachment was further elucidated by examining the effects of heparinase. This enzyme which specifically hydrolizes HS, led to neuronal detachment in vitro and in vivo during the early stage (2-3 days) of neuronal growth. Established cultures were not affected by heparinase. We conclude: 1) the expression of astroglial invasive activity can be altered and may be dependent upon the state of differentiation, and 2) HS is probably involved in the formation of neuron-substrate contacts.
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THURSDAY AM

406.13 REGULATION OF PHOSPHATIDYLCHOLINE SYNTHESIS BY PHOSPHATIDYLCHOLINE AND HEPARIN IN MICROGLIA CELLS. K. J. S. Black, J. K. Black, and J. H. Wurtman. Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge, MA 02139.

Cultured microglia cells (LA-N-2) incubated in phosphatidylcholine (PC) showed increased incorporation of [14C]choline into phosphatidylcholine (PC). The effects of PC and heparin were additive in several cell types by phospholipase A2 and diacylglycerol, both of which activate the calcium and phospholipid-dependent enzyme, protein kinase C. Phosphatidylcholine (PC) or heparin (DiC8, 1M) increased PC synthesis by 14% and 50%, respectively, in untreated LA-N-2 cells. In cells previously exposed to PS, PM and DiC8 stimulated incorporation of [14C]choline into PC by approximately 35% and 120%, respectively, compared to cells treated with PS alone. The effects of PS and DiC8 were additive, together these compounds increased PC biosynthesis by 60%. Under these conditions, the total increase in PC synthesis in presence of untreated controls was over 4-fold. In preliminary experiments, choline phosphatidyltransferase activity was increased in cells prelabeled with PS (10 to 30 min). It remains to be determined whether PS treatment will also stimulate the role of enzyme diacylglycerol. The results suggest that PS may stimulate PC synthesis by potentiating the activation of protein kinase C by phosphorylation of diacylglycerol. Our findings also indicate that formation of PS within the membrane may constitute a physiologic stimulus to overall membrane biosynthesis.

406.14 EFFECT OF CHRONIC ADMINISTRATION OF DIGITOXIN ON RETINAL MEMBRANE Ca2+-Mg2+ ATPASE. M. L. Michaelis, T. E. Klock and R. A. Sohatz, Pharmacology, Univ. PR School Med. San Juan, PR 00936.

The rat retinae at the age of 34 days were perfused with digoxin (0.1-1000 mg/kg) via the carotid artery for 2 hours. Na,K-ATPase was measured in the retinae from untreated controls and in digoxin-treated animals. Incorporation into a broad group of proteins ≤58 kDa was unaffected. The results demonstrate that synthesis of the catalytic subunit can be differentially regulated and that chronic administration of digoxin in a clinical setting may alter the usual cellular composition of Na pump isofoms.
DIFFERENTIATION AND DEVELOPMENT VI

407.1 EXPRESSION OF TYROSINE HYDROXYLASE (TH)-IR AND mRNA IN RAT CHOLINERGIC CILIARY GANGLION NEURONS. S. Lantos, A. Acherson and K. K. Siegel. Center for Neuroscience, Case Western Reserve Univ., Cleveland, OH. 44106.

The ciliary ganglion (CG) is classified as parasympathetic based on several criteria including location, acetylcholine synthesis and cholinergic target effects. Some CG neurons may contain neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) and are present in the adult paravertebral ganglion (PG) and in axons in the adult urogenital nerve. Acetylcholine (ACHE) and NADPH-diaphorase are demonstrable in the PG on postnatal day 0. At this time, TH and VIP have been measured in the CG and sympathetic cell bodies. A high molecular weight band was also detected in CG. In situ hybridization and immunohistochemistry have been performed. Comparison of immunoblots of CG and sympathetic ganglia revealed a 62kd band in both tissues. Analysis of TH neuronal density by using a similar amount of TH mRNA per cell. By analogy with TH regulation in sympathetic neurons, NGF and impulse activity are likely candidates to mediate these effects. Supported by NICHD 23678.


Our lab is currently interested in the development of the trigeminal, enteric, and sympathetic nervous systems. Acetylcholine (ACH) is a marker for cholinergic neurons, tyrosine hydroxylase (TH) a marker for noradrenergic neurons, neuropeptide tyrosine (NPY) and vasoactive intestinal polypeptide (VIP) are present in the adult paravertebral ganglion (PG) and in axons in the adult urogenital nerve. ACH, TH, NPY and VIP are cell bodies and terminals in the PG on postnatal day 0. At this time, ACHE, TH, NPY and VIP immunoreactivity is also seen in the cervix, mainly as bundles of preterminal axons. At early stages of development, ACH and NPY fibers are not apparent but appear distinct by day 36. ACHE staining appears to increase in intensity in neurons and axons from days 0 to 36. There appears to be a relative decrease in the number of TH neurons and axons from day 36 to mature weights. A relative number of VIP and NPY neurons and axons increases from day 0 to 16. During this time NPY and VIP axons begin to branch, and form a mature plexus by day 36. Supported by NIH Grant NS-22526.


The mechanisms underlying the generation of phenotypic diversity in enteric neurons are unknown. Serotonergic and cholinergic neurons appear earlier in ontogeny (day E 12) than those which contain substance P or VIP (day E 14). Although substantial stores of endogenous 5-HT and neural 5-HT receptors are acquired after neonatal neurotrogeny can be first recognized. Enteric neurone precursor cells divide in the presence of mature neurons. These observations have led to the hypothesis that peptide neurons develop later than those that utilize 5-HT as a neurotransmitter. Early developing neurons affect neural development. In order to test these hypotheses, we studied the phenotypic expression of neurons recognized by antisera to NPY and CORP, and by the histochemical NADPH-diaphorase. NPY immunoreactivity (IR) appeared for the first time on day E 12, when it was seen in the stomach. NADPH-diaphorase, which is co-expressed with NPY-IR in many enterochromaffin cells by day E 13, the entire length of the bowel contained NPY-IR. In contrast, CORP-IR could not be detected anywhere in the gut until the end of day E 14, all by day E 18. The NPY/CORP-IR diaphorase staining of neurons that develop before the acquisition of a detectable level of endogenous 5-HT or enteric neural 5-HT receptors. Conversely, small molecule transmitters do not necessarily develop earlier than peptides in enteric neurons and it is unlikely that 5-HT affects the differentiation of NPY/CORP-diaphorase neurons. Nevertheless, the possibility that 5-HT or another enteric neurotransmitter affects CORP-containing neurons has not been excluded. Supported by NIH grants # NS 22837, NS 15547, and NS 12969.

407.6 SPATIAL AND TEMPORAL EXPRESSION OF NEUROPEPTIDE mRNAs ENCODING MET-ENK, POMC, AND TRH. MAPPING IN Xenopus laevis USING IN SITU HYBRIDIZATION. W. P. Hayes, R. T. Zoller and Y. F. Leb Linnich, NCINCNID, NIH, Bethesda, MD. 20892.

Neuropeptides have recently been implicated as trophic factors in neuronal development in addition to their endocrine effects. To examine this possibility in early development, we have turned to the frog as a model system. In situ hybridization histochemistry was used to detect neuropeptide mRNA expressing cells in adult and developing Xenopus laevis. Due to low amounts of these mRNAs, in situ hybridization with specific antibodies indicated that the expression of these mRNAs is differentially controlled and related to the various phases of cell proliferation and synaptogenesis during brain development.
DIFFERENTIATION AND DEVELOPMENT VI
THURSDAY AM

407.7
THE SUBSTRATE INFLUENCES NEUROTRANSMITTER EXPRESSION IN CULTURED AVIAN DORSAL ROOT GANGLION CELLS.

407.8

We determined the relationship between expression of neuropeptides in culture and the age of the fetuses from which the neurons were derived. Neurons derived from the celiac ganglion-extended cultures from 3-day-old chick embryo, plated on poly-L-lysine in DMEM+10% FCS; and b collagen as culture substrate was prepared and stained by immunocytochemistry.

In this study, assessment of the same parameters, expressed as ng/mg protein/h, for cultures on collagen revealed that while CHAT was not affected by the different substrata and thus the different cell organisation, GAD was always markedly higher, increasing from culture day 0 to 0.17 and reaching a peak on day 14. Similar effect was produced by long NGR/ml of culture medium for neurons grown on poly-L-lysine.

Immunostaining for GABA and GAD revealed the presence of neuronal and non-neuronal immunoreactivity. In all respects (phenotypic characteristics of neurons), the cultures closely mimics that observed in vivo. This model system may allow an examination of factors influencing CA neurotransmitter phenotype expression in the developing CNS. (Funded by NSF BNS-851079 and NIH RR-08139.)

407.10
AMOUNTS OF TYROSINE HYDROXYLASE mRNA DURING MATURATION OF RAT HYPOTHALAMUS, SUBSTANTIA NIGRA, SUPERIOR CERVICAL GANGLION AND ADRENAL. W. Kedzierski* and J.C. Porter, Dept. OB/Gyn and Physiol., Univ. of TX Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

The purpose of this study is to investigate the effect of age on tyrosine hydroxylase (TH) mRNA in catecholamine-secreting cells. TH mRNA was analyzed by an SI nuclease assay utilizing a [32P]-labeled RNA probe (cRNA) that is complementary to TH mRNA. TH mRNA was extracted from tissues (cerebral cortex, hypothalamus, substantia nigra, and adrenal) and purified by urea PAGE. An autoradiogram of the gel was analyzed by densitometry. TH sense RNA, which served as a standard, was treated similarly. TH mRNA increases five to sixfold between 4 days and 6 weeks of age in the hypothalamus, substantia nigra, and adrenal (Table 1).

Further development does not change the level of TH mRNA in the brain. However, between 6 weeks and 12 weeks, TH mRNA in the adrenal increased twofold.

Table 1. TH mRNA (amoles) in maturing male rats.

<table>
<thead>
<tr>
<th>Age</th>
<th>thalamus</th>
<th>nigra</th>
<th>ganglion</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>7.5 ± 0.4</td>
<td>107 ± 2</td>
<td>--</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>6 days</td>
<td>45 ± 3.0</td>
<td>107 ± 4</td>
<td>21 ± 1.4</td>
<td>378 ± 21</td>
</tr>
<tr>
<td>12 days</td>
<td>51 ± 0.8</td>
<td>478 ± 33</td>
<td>274 ± 11</td>
<td>865 ± 30</td>
</tr>
</tbody>
</table>

407.11

Avian dorsal root ganglia (DRG) contain a population of cells that are able, when cultured in vitro, to express a catecholaminergic phenotype, displaying tyrosine hydroxylase (TH) immunoreactivity and a synthesis of noradrenaline (Xue, Z.G., et al., J. Neurochem. 50:142, 1988) reveals that TH mRNA synthesising noradrenaline (Xue, Z.G., et al., Proc. Natl. Acad. Sci. U S A 85:4071, 1988) reveals that TH mRNA was expressed as nmole/mg protein/h for cultures on collagen. Thenceforward, the cells, their emergence from dividing precursors, the kinetics of expression, the regulation of TH mRNA and protein, the catecholamine differentiation observed in the presence of insulin appears identical to that occurring in medium containing CEE. We suggest that insulin-like polypeptides are plausible candidates for a role in triggering the normal development of sympathetic precursors in vivo.

407.12

Numerous TH-immunoreactive cells occur in the embryonic spinal cord, both ventral to the central canal and along the lateral border of the dorsal horns (Xue, Z.G., et al., Neuroscience, 38(1), 1993). While only occasional cells are detected on embryonic day 15 (E15), these cells quickly reach their full complement in numbers by E17. A [32P]cDNA probe was used to examine development of the TH-positive cells grown in vitro. Dissociated cells from chick spinal cords from E8 or E10 embryos were incubated for varying lengths of time on collagen-coated plastic slides.

When cultured for 8 days, large numbers of TH-immunostained cells are present. Cells are also detected by anti-DA immunocytochemistry, although their number represents less than 10% of TH-stained neurons found in sister cultures. When the cultures are incubated for longer periods (approximating embryonic in vivo ages earlier than very few TH–positive cells are detected in vitro, although relative timing of appearance of TH-positive cells in vivo closely mimics that observed in vivo. This model system may allow an examination of factors influencing CA neurotransmitter phenotype expression in the developing CNS. (Funded by NSF BNS-851079 and NIH RR-08139.)
DIFFERENTIATION AND DEVELOPMENT VI

407.13

ONTGENY OF THE PROTHORACICOSTROPHOPES IN THE TOBACCO MOTH, MANBUCA SEXTA. A. L. Bennett, J. E, Bollenbacher, Dep. of Biology, CB 3280, Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280.

Prothoracicotropic hormone (PTTH) is a neurohormone which regulates insect molting and metamorphosis. The expression of PTTH during embryogenesis in Manduca sexta was examined immunocytochemically using a monoclonal antibody against PTTH. The neuroepithelium was first expressed by the 4 lateral cerebral neurosecretory cells (L-NSC III) between 25-30% of embryonic development. The developing axons of these nerves traversed the cerebral lobes and decussated to the contralateral lobe approximately halfway through embryonic development, and reached the brain via the nerve corpora cardiale I (approximately 60% development). Growth of dermotic processes within the protocerebral neuropil began at approximately 60% development, and their complexity increased up to hatching. By approximately 75% embryonic development, the axons had reached the corpora allata and arborized forming the terminal varicosities of the neurohemal organ. In addition to the L-NSC III, transient PTTH-like immunoreactivity was observed in the ventral and subesophageal ganglia, as well as the lateral brain regions.

407.14

EARLY DEVELOPMENT OF GABA IMMUNOREACTIVE NEURONS IN CEREBRAL CORTEX OF FETAL MONKEYS. J. W. Harper, J. I. Jenkins, H. H. Kilander, L. Chalupa and E. G. Jones, Dep. of Anatomy, Yale University School of Medicine, New Haven, CT 06510.

Many features of cortical connections in monkeys are well specified by the last third of gestation. Here, we present a preliminary report of the development of GABA-containing local circuit neurons in the macaque prefrontal cortex (PFC). Sections of dorsolateral PFC from 15 fetal monkeys were processed for GABA (magnetically immunoreactive) at 24 hours after origination of the 165 day gestational period. GABA-immunoreactive neurons (GNs) are present within the cortical plate (CP), the underlying subplate (SP) and the intermediate zone (IZ). The majority of GNs form a band lying within the lower layer VI and the upper portion of the SP. GPs/GN neurons are multipolar or have an elongated soma with leading and trailing processes, the latter resembling migrating cortical neurons. Between E30 and E72 the majority of GPs/GN neurons increases and cells in the subplate and lower layer VI develop elaborate processes. GPs/GN are now present throughout the CP; their density increasing from superficial to deep with the exception of the high density in the marginal zone. Between E72 and E116 their density in the IZ/SP diminishes. At E116 all GPs/GN are non-pyramidal in morphology and are denser in layers I and VI. The E116 pattern is not fully mature in that relatively few labeled cells are found in layer II and upper layer III. Between E116 and E131 two dense bands of labeled cells emerge: one spanning layers I, II and upper layer III and the other in layer IV. GPs/GN in the emerging white matter are now fewer in number and the overall laminar pattern is similar to that of mature monkeys. These results indicate that many neurons of the developing cortex express GABA within the first third of gestation. Further, their morphologic and early appearance in the IZ and SP suggest that GABA may be expressed in some neurons during migration. Supported by BNS817585-MH38546 and NS22607.

407.15

LAMINAR DISTRIBUTION OF GABA RECEPTOR AND GABA IMMUNOREACTIVITY DURING DEVELOPMENT IN THE MAMMALIAN CEREBRAL CORTEX. A. Cobas, G. Alvarez-Solado, D.L. Blas and I. Fairén, ISPN European Neuroscience Association, Instituto Cajal, CSIC 28006 Madrid, Spain, and Department of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Rats aged from E16 through P33 were processed for the immunocytochemical localization of GABA (Seguela et al., 1984, EM 888-921) and GABA receptor (Cobas et al., 1984, J. Neurosci. 8:1102-14; Vitorica et al., 1984, J. Neurosci. 8:675-221). At E16, GABA* cells are abundant in the lower intermediate zone and at both sides of the cortical plate (CP), which is also outlined by two narrow bands of GABA* immunoreactivity. With advancing development, the marginal zones soon become the most densely immunoreactive lamina for the GABA receptor. In periatal neurons, the neuropil between the inferior border of the CP and the lamina of GABA* immunoreactivity characterizes laminar differences in intensity. GABA* cells are abundant at this level. The lamina of GABA* immunoreactivity gradually becomes less distinct during the next stages of postnatal development. At P33, the densest GABA* immunostaining is in layer I, the rest of the layers showing a rather uniform pattern of staining. With the exception of the earliest stages, a parallelism exists between the laminar patterns of GABA* and GABA* immunoreactivities observed during cortical development.

407.16


The goal of this work has been to develop an amphibian model for the growth of capillaries of the CNS. Anesthetized tadpoles of albinos Xenopus laevis are sufficiently transparent that individual capillaries, their early sprouts and sprouts can be resolved on the surface of the optic tectum in vivo by confocal light microscopy.

1. Early development indicated the pial capillaries developed by the classical mechanism of sprouting of endothelial cells from existing blood vessels (Eldon Clark, 1918).

2. "Deep sources" apparently developed from surface capillary sprouts that generated the basement membrane, invaded the nervous tissue, and joined more arterioles in the ventral brain.

3. The dorsal medial vessels enlarged in diameter as they drained an increasing blood flow from the tectum into the sinuses of the caudal choroid plexus.

4. Some capillaries disappeared during development.

5. No significant differences in densities of surface vessels or of deep sources were observed after removal of one eye, rearing at 30° or 12°, or rearing at half atmospheric pressure or in high O2.

407.17

TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND DOPAMINE SYNTHESIS PRECEDE DOPAMINE UPTAKE IN MESENCEPHALIC DOPAMINERGIC NEURONS FROM RAT EMBRYOS. M.L. Finneman, A. Zuddas, J. Barker and U. di Perio. Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

In this report we show that in vivo development of tyrosine hydroxylase (TH) immunoreactive and dopamine (DA) neurones within the mesencephalic DA neurones from rat embryos takes place before the onset of DA uptake system. Ventral mesencephalon from embryonic day 11 (E11) to 1 week after birth were dissected and frozen for determination of endogenous TH, TH immunoreactivity and dopamine (DA) neurones. TH immunoreactivity and DA neurones were located in the dorsal mesencephalon and were similar to those in the adult, and show significant changes in morphology, distribution and density over the gestational period examined. Supported by Grants EY 06432, NS 21377 and RR 00169 from the National Institutes of Health.

407.18


The frontal lobes from six fetal rhesus monkeys (Macaca mulatta) were used to examine the morphology and distribution of immunocytochemically identified GABA and neuropeptides containing neurons and to study the distribution of tyrosine hydroxylase-immunoreactive (TH) fibers during the third trimester of gestation (E110,121,131,135,150,155). All ir-cell and fiber populations were present from the youngest age. In each of the fetuses, GABA-ir neurons were present in all layers and in the subcortical white matter, but were denser in a superficial band (E110,121,131,135,150,155). Neurons for NPY and SP were scattered throughout layers II-IV, but were denser in the underlying white matter. Neurons for NPY and SP were scattered throughout layers II-IV, but were denser in the underlying white matter. Neurons for NPY and SP were scattered throughout layers II-IV, but were denser in the underlying white matter. Neurons for NPY and SP were scattered throughout layers II-IV, but were denser in the underlying white matter.

The presence of a prodynorphinergic striatonigral pathway and the presence of kappa opiate receptors in the substantia nigra (SN) suggest a physiological action of kappa-selective peptides in this area. In order to investigate specific kappa actions in the SN, the selective agonist, USO4,488H, was applied by microliontophoresis during extracellular single unit recording of neurons in the SN para compacta (SNC) and the SN para reticulata (SNR) in cats.

USO4,488H appears to affect dopaminergic neurons of the SN and predominantly inhibited the spontaneous firing of neurons in the SNR. Approximately 50% of SNR neurons tested were inhibited by USO4,488H, and this effect was dose dependent. USO4,488H appears to differentially affect SNR neurons that are distinguished by their response to a mechanical pressure stimulus applied to the hindpaw. Cells that exhibited an increase in firing rate in response to mechanical pressure are inhibited by USO4,488H more often than cells that exhibit a decrease in firing in response to the stimulus or that are unaffected. These results support previous findings from this laboratory that the systemic administration of USO4,488H differentially inhibits SNR neurons that exhibit excitation in response to mechanical pressure.


We have reported a population of neostriatal neurons containing tyrosine hydroxylase-like immunoreactivity (TH-LI) (Neurosci Lett 75:205). We have now found these cells to be positive for dopa decarboxylase, but not for dopa decarboxylase in this area. In some monkeys, however, we have shown fewer positive cells than in others. To examine such variability while minimizing technical variations in processing, we performed immunohistochemistry on tissues from six animals simultaneously, treating comparable sections from each of twelve hemispheres together in each container. Results indicate a wide range of variability among individuals. In several animals, including three of those injected with 6-OHDA, the distribution of several different receptor types that bind neurotransmitters is now under investigation. (USPHS grants NS19237 and RR01166).

408.4 SUBSTRATE DISTRIBUTION OF THE BASAL GANGLIA OF PIGEONS. E.K. Richter, L. Ablin, A. Reiner, A.B. Young, and J.B. Henegy, Jr. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI and Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN.

Recent studies have shown that the basal ganglia in birds consists of fundamentally the same neuronal populations, with the neurons of each population being definable in terms of their connections and neurotransmitter content. These results have suggested a high degree of similarity in functional organization of the basal ganglia among birds and mammals. To further explore this, we have used a toxicologic and immunohistochemical approach to examine the pigeon basal ganglia for the presence and distribution of several different receptor types that bind neurotransmitters known to be present in avian basal ganglia and their projection systems.

The following receptor types were examined: 1) D1 dopamine (SCH23390); 2) D2 dopamine (piperizine); 3) mus匠心 cholinergic (GABA); 4) NMDA (glutamate blocked with cold NMDA); 5) GABA-A (GABA blocked with cold baclofen); 6) GABA-B (GABA blocked with cold isoguvacine); and 7) benzodiazepine (BDZ) (flunitrazepam). The stratum was found to be rich in D1, D2, muscarinic, NMDA, GABA-A, and BDZ receptors. Of the target areas of the stratum, the pallidum was rich in GABA-A and BDZ receptors, while the nigra contained moderate levels of D1, D2, muscarinic, GABA-A and BDZ receptors. These results are similar to those in mammals and are consistent with the view that neurotransmitters previously localized to the pigeon basal ganglia play roles very similar to the roles they play in mammals. Supported by NS19620 (A.R.) and NS19613 (A.B.Y.).

408.5 DESCENDING PROJECTIONS FROM SUBSTANTIA NIGRA TO THE MEDULLARY RETICULAR FORMATION: R. von Economo* and A.D. Smith. MRI Anatomical Neuropathology Unit, University Dept. Pharmacology, South Parks Road, Oxford, OX1 3QT, UK.

We used both retrograde and anterograde neuroanatomical tracers to report a descending projection from the substantia nigra to the medullary reticular formation of the rat. The rat's retrograde tracer Wheat Germ Agglutinin Horseradish Peroxidase (1-5%, Sigma) was injected (8-100 nl) into the lateral parvocellular reticular formation. These injections revealed the presence of retrogradely labelled cells in both substantia nigra par compacta and par pars compacta, respectively, with the majority of cells found in the substantia nigra compacta. Within the substantia nigra, labelled cells were restricted to the caudal lateral half of the substantia nigra. Injection of the anterograde tracer Phaseolus Vulgaris Leucagglutinin into the caudal and lateral substantia nigra revealed a medullary projection which was restricted to the lateral parvocellular reticular formation, FCRT. This anterograde labelling ran throughout the rostrocaudal extent of the FCRT. Also labelled was the rostral region of the nucleus of the solitary tract, NTS. Caudally, the labelling in the NTS was restricted to the ventrolateral NTS, dorsally adjacent to the FCRT.

408.6 SUBTRACTIVE cDNA CLONING OF NEURONALLY EXPRESSED mRNAs RICH IN RAT CAUDATE PUTAMEN. Joseph B. Watson* and J. Gregory Sutcliffe (SPON: S. Forss-Petrill). Molecular Biology Department, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Precise knowledge of the neuronal organization of the neostriatum (caudate nucleus, putamen) will help to better define its role in voluntary movement and may suggest hypotheses to explain striatal pathologies (e.g. Huntington's Disease). One important task is to describe the presently known striatal cell types in more molecular detail. Toward this end, we are systematically isolating cDNA clones (such as PCR of mRNAs with in rat caudate putamen). PCRT detects two postnatally expressed mRNAs (1.0 kb, 1.5 kb) prevalent in striatum, cerebral cortex, hippocampus, much less abundant in olfactory bulb, poas, and undetectable in cerebellum. Translation of a full length cDNA sequence predicts a novel 9.4 kDa protein with a cysteine-rich domain at its N-terminal. This domain is similar to that of ovine thyroglobulin and Cs5a phalvolaxton, and a glycine-rich domain at the COOH-terminus similar to that found in keratin. In situ hybridization experiments show that RC3 mRNA is expressed in restricted neuronal populations of the telencephalon and diencephalon. There is elevated expression in medium-size neurons of the caudate putamen over globus pallidus suggesting that RC3 may be a marker for a class of striatal neurons. Analysis of additional cDNA clones detected by subtractive hybridization using a rat caudate putamen enriched cDNA probe will also be presented.
040.7 EFFECTS OF INTRASTRATIAL ESTROGENS ON THE DORSAL IMMUNOREACTIVE IMMUNE CELLS IN CANCER PATIENTS C. VAN HARENVELT, C. A. OTTRELL, AND C. M. BERRY. Psychology Dept., U. Florida, Gainesville, FL 32611. Previous research has shown that in ovariolectomized rats, estrogen injection into the hippocampus evokes a rapid and prolonged improvement in spatial memory. In this study, we examined the effects of estrogen on the immune system of rats. We injected estradiol benzoate (EB) and progesterone (P) into the ventral hippocampus of rats and measured the number of immune cells in the brain. The results showed that EB alone did not affect the number of immune cells, but when combined with P, it increased the number of immune cells in the hippocampus. This suggests that estrogen and progesterone interact to elicit an immune response in the hippocampus.

040.8 POTENTIATION OF THE DORSAL IMMOBILITY RESPONSE BY HORMONES IN THE BASAL GANGLIA OF MALE AND FEMALE RATS. M.E. Mayer, * G.A. Cottrell and C. Van Harenvelt. Psychology Dept., U. Florida, Gainesville, FL 32611. The dorsal immobility response (DIR) is a species-typical inhibitory response that is elicited by grasping the rat near the base of the neck. The DIR persists if the rat is not held in place. We administered hormones directly to the basal ganglia of male rats to determine their specificity and size of action on this response.

040.9 EFFECTS OF INTRASTRATIAL ESTROGENS ON THE DORSAL IMMUNOREACTIVE IMMUNE CELLS IN CANCER PATIENTS C. VAN HARENVELT, C. A. OTTRELL, AND C. M. BERRY. Psychology Dept., U. Florida, Gainesville, FL 32611. Previous research has shown that in ovariolectomized rats, estrogen injection into the hippocampus evokes a rapid and prolonged improvement in spatial memory. In this study, we examined the effects of estrogen on the immune system of rats. We injected estradiol benzoate (EB) and progesterone (P) into the ventral hippocampus of rats and measured the number of immune cells in the brain. The results showed that EB alone did not affect the number of immune cells, but when combined with P, it increased the number of immune cells in the hippocampus. This suggests that estrogen and progesterone interact to elicit an immune response in the hippocampus.

040.10 NUCLEUS OF THE POSTERIOR COMMISSURE IN THE RED EARED TURTLE, PSEUDEMYS SCRIPTA. P. M. Bell and P. S. Ulinski, Dept. Anatomy, University of Chicago, Chicago, IL 60637.

040.11 THE SUBSTANTIA NIGRA OF THE RED-EDARED TURTLE, PSEUDEMYS SCRIPTA ELEGANS. A. F. Chang* and P. S. Ulinski, Dept. of Anatomy, University of Chicago, IL 60637.


The substantia nigra gives rise to a dopaminergic projection back to the striatum, but nothing is known about the functional architecture of this projection in reptiles. Thus, we investigated the localization of CGRP-immunoreactive axons in the substantia nigra of turtles. The substantia nigra is divided into two zones, the pars compacta and the pars reticulata, and is involved in the control of locomotor behavior. The CGRP-immunoreactive axons are localized in the pars compacta and pars reticulata, and are concentrated in the midline and lateral parts of the substantia nigra.
408.13

SPINDLE ACTIVITY IN THE THALAMUS IN VITRO.

M. Seraphin* and M. Lübbersholtz, Dept de Physiologie CNU, 1211 Genève 4, Switzerland.

Intracellular recordings from thalamic neurons have been obtained so far in situ in the cat and in guinea pig slices. However in order to study membrane properties within a complex circuitry, alternative preparations must be used. We therefore recorded from thalamic neurons in an isolated whole brain preparation (IWB) and found that they displayed all the properties already described in slices. However they displayed in addition spindle activity either spontaneously or in the presence of barbiturates. These spindles were very much the same than those recorded in the intact cat. They were indeed characterized by a spindle duration of 1-3 sec. consisting of low threshold rebounds and spikes and the interspindle interval was 10-20 sec. These spindles could be manipulated either by barbiturates stimulations or by the application of drugs in the perfusion.

Our studies indicate that thalamic circuits are preserved in the IWB which could thus be used for studying intrinsic thalamic mechanisms as well as intercortical interactions.

(Supported by a Swiss NSF grant no 3.288-0.85)

408.15

THE SUBSTANTIA NIGRA PARS RETICULATA PROJECTION TO THE SUBTHALAMIC NUCLEUS OF THE RAT. H. Kaiser* and T. Kiel, Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, Tennessee 38163.

Projection from the substantia nigra pars reticulata (SNr) to the subthalamic nucleus (STH) was studied in rats using the PHA-L anterograde tracing technique. PHA-L was injected into the SNr or into the nigrostriatal tract. After 2-3 days of survival, animals were fixed and the brains sectioned on a vibratome. Sections were immunocytochemically stained using anti-PHA-L antibodies. The results demonstrate that the STH receives a major projection from the SNr. The labeled axons stain for both dopamine and GABA containing markers. Electron microscopic analysis of the labeled terminals in the STH revealed that the labeled boutons were large and contained many spherical or ellipsoidal vesicles and a number of mitochondria. Most of the labeled terminals were synaptic with dendritic shafts. These morphological features were very similar to the GABA-immunopositive boutons in the STH. The observations indicated an existence of probably GABAergic projections from the SNr to the STH. This newly found projection may function as a negative feedback circuit of previously demonstrated excitatory STH projection to the SNr (Nakajima et al., Brain Res.). Supported by NIH grants NS 20702 and NS 23886 to STK and NS 25783 to HK.

408.17

QUINOLINATE AND KAINTATE NEUROTOXICITY IN NEOSTRIATAL CULTURES IS POTENTIATED BY CO-CULTURING WITH NEOSTRIATAL NEURONS. F. Galmeraz* and J.D. Sumpter and T.N. Kish (SPON: T. Gardiner), Dept. of Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, Tennessee 38163.

There is a substantial loss in glutamate neurotoxicity in the neostriatum following cortical deafferentation (Bixiere and Coyle, J Neurosci 7: 2388-2394, 1987). In order to study the mechanisms by which this loss occurs, we have examined the effects of quinolinate or kainate on cultures of neostriatal neurons. We found that the death of neurons in cultures treated with quinolinate or kainate was potentiated when they were co-cultured with cortical neurons. We have used a variety of methods to study this potentiation. We used Scatchard plots, and Hill plots to analyze the data. We found that the Hill coefficients for both substances to neostriatal neurons was potentiated when they were co-cultured with cortical neurons. In particular, the Hill coefficient for quinolinate was increased 2-fold by the presence of cortical neurons. These results suggest that different mechanisms are responsible for the toxicity of these glutamate analogs. Preliminary immunocytochemical studies indicate that different neuronal phenotypes are affected by quinolinate and kainate as well. Supported by NIH grants NS 20702 and NS 23886 to STK and a Huntington’s Disease Foundation grant to DJJ.

408.18

MUSCARINIC RECEPTORS IN PRIMARY CULTURES OF RAT NEOSTRIATUM. P. T. Akins*, D. J. Surmeier, B. T. Kish (SPON: C. M. Blatiaux), Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, Tennessee 38163.

We have characterized some pharmacological properties of the receptors expressed on intact striatal neurons. Cultures were generated from fetuses at 17 days gestation. After dissociation and mechanical dissociation, cells were plated into 12 well dishes at 2000 mm2 for neuronal cultures or 200 mm2 for glial cultures. Cultures were fed serum-supplemented DMEM/F12 for 14-16 days.

Binding studies were carried out in HEPES buffered Hank’s balanced salt solution (HBSS) at 23 degrees C. Cultures were preincubated for 15 min. Binding site labeling was achieved by incubation for 45 min. with 0.15 nM [3H]-(+)-acetylcocholine ([3H]-ACh) ranging from 0.01 to 2 nM. Cell counts were made from representative identified fields before and after (19-24 hr) radioactivity addition. Cultures were expressed as a percentage of the number of cells originally present. In order to distinguish cortical from striatal neurons in co-cultures, cortical neurons were labeled with the fluorescent carbocyanine dye DII during dissociation. Cortical neurons could be readily identified for several weeks with this procedure.

Our results indicate that quinolinate is neurotoxic to striatal neurons cultured in the absence of cortex. However, the neurotoxicity of both substances to neostriatal neurons was potentiated when they were co-cultured with cortical neurons. D-2-aminosuccinic acid (D-ASA), a competitive antagonist for quinolinate, did not significantly reduce the effects of KA, suggesting that different mechanisms are responsible for the toxicity of these glutamate analogs. Preliminary immunocytochemical studies indicate that different neuronal phenotypes are affected by quinolinate and KA as well. Supported by NIH grants NS 20702 and NS 23886 to STK and a Huntington’s Disease Foundation grant to DJJ.

408.14

ELECTROPHYSIOLOGY OF ENCEPHALITIC NEURONS AND THEIR RESONANCES TO SUBTALUMAL STIMULATION IN PRIMARY BRAIN SLICE PREPARATIONS. H. Nakajima* and T. Kishi (SPON: T. Kishi), Dept. of Anatomy & Neurobiology, The University of Tennessee, Memphis, TN 38163. Supported by NIH grants NS 20702 and NS 25783 to HK.

It is well demonstrated that single subthalamic (STH) neurons in the rat project both to the pallidum and to the substantia nigra (SN). Our previous in vivo study demonstrated that stimulation of the STH evoked EPSPs in SN neurons. In this study, we examined electrophysiology of encephalitc (EP) neurons in the rat brain slice preparation. STH stimulation was performed with bipolar stainless steel electrodes. EP neurons were classified into two groups based on their membrane properties. Type I neurons, which formed a major group, were characterized by having spontaneous repetitive firing and relatively short (less than 100 ms) spike after-hyperpolarization. Type II neurons, in contrast, had no spontaneous firing and the spike triggered by intracellular stimulation was followed by a long (more than 200 ms) after-hyperpolarization. In type-I neuron, stimulation of the STH evoked short latency monosynaptic EPSPs and bicuculline-sensitive IPSPs. Based on already known physiological and anatomical evidences, it is likely that these monosynaptic EPSPs originated from the STH. Supported by NIH grants NS 20702 to STK and NS 25783 to HK.
BASAL GANGLIA AND THALAMUS: MOTOR SYSTEMS V

408.19
MEMBRANE PROPERTIES OF PEDUNCULOPONTINE NEURONS AND THEIR RESPONSES TO NIGRAL STIMULATION IN AN IN VITRO SLICE PREPARATION. Y.N.Kane*, S. Afsharpour, and S.T.Kita, Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

Electrical membrane properties of neurons in rat pedunculopontine nucleus (PPT) and their responses to substantia nigra reticulata (SNr) stimulation were studied in parasagittal brain slices (400-500 µm) which contained the brachium conjunctivum (BC), substantia nigra and the subthalamic nucleus (STh). Intracellular recordings were obtained using conventional techniques. SNr was stimulated by three bipolar stimulating electrodes. In some experiments, a pair of additional stimulating electrodes was placed in the BC or between STh and SNr. Twenty out of 80 recorded neurons were intracellularly injected with biocytin. Slices were fixed with 4% paraformaldehyde, immersed in 20% sucrose, frozen and sectioned at 50 µm and stained with avidin–Texas Red for fluorescent visualization.

These sections were then processed immunocytochemically for ChAT or counterstained with cresyl violet. All retrogradely labeled neurons were located in the PPN.

Electrical membrane properties of PPN neurons were studied by intracellular current injection through the recording microelectrode. The results indicated that PPN neurons may be classified into several categories based on their electrical membrane properties such as prominent afterhyperpolarization, TTX-sensitive outward currents, TTX or cobalt sensitive inward currents and high threshold TTX insensitive spikes which were blocked by cobalt.

Responses to SNr stimulation were predominantly monosynaptic IPSPs. It was frequently observed that IPSPs were followed by robust Na spikes possibly generated by two types of slow inward currents. Occasional EPPSs were seen following SNr stimulation and were considered to be due to the activation of passing fibers in SNr since stimulation of STH or the area between STH and SNr produced EPPSs with similar shape but with slightly longer latencies. Supported by NIH grants NS 20702 and NS 23886 to STK.

408.21
PATTERNS OF TERMINATION OF RAT BASAL GANGLIA AND CEREBELLAR EFFERENTS IN THE THALAMUS: STRICTLY SEGREGATED AND PARTIALLY OVERLAPPED PROJECTIONS. J.M. Denton*, H. Kakei S. Popoff, and D. Desimone, Dept. of Anatomy & Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

"Lab. de Physiol. des Centres Nerveux, Univ. Piere et Marie Curie, F-75230 Paris Code 05, France.

There is a widely held view that basal ganglia and cerebellar outputs act independently via separate subcortical channels. In this study, the patterns of innervations provided by the deep cerebellar nuclei (CDN), entopeduncular nucleus, and the substantia nigra pars reticulata were reinvestigated in rats using the PHA-L anterograde tracing technique.

PHA-L was iontophoretically injected into these brain areas. After two weeks of survival, animals were perfused fixed and the brains sectioned on a vibratome. Sections were immersed in 10% saline and 1% formaldehyde. Tissue sections were reacted with avidin–Texas Red for fluorescent visualization. Sections were immunolabeled for TH+ neurons with secondary antibodies conjugated to FITC. Preliminary results indicate that double-labelled biocytin and TH+ neurons can be readily demonstrated with this procedure. Results also suggest that there is a significant probability of impinging non-dopaminergic neurons in the SNr. Supported by NIH grants NS20702 and NS23886 to STK and HS 2783 to HK.

409.1

By embryonic day 16 in the rat, separate cells in the substantia nigra have developed ipsi- and contralateral axonal projections to the striatum. Retrograde fluorogreen tracer (Fast Blue (FB)) injections into the striatum during the late embryonic and early postnatal period, revealed that both the ipsi- and contralateral nigrostriatal projections undergo marked periods of perinatal cell death. However, the time courses of cell death were different. By postnatal day 1, 85% of the embryonic population of contralateral axonal projections to the striatum were undergoing marked periods of perinatal cell death. In contrast, the number of striatal projecting neurons in the much larger ipsilateral pathway increases over the same time period, projecting nigrostriatal neurons die. In contrast, the number of striatal projecting neurons in the ipsilateral pathway increases over the same time period, whereas the number of striatal projecting neurons in the contralateral pathway decreases. This study supports the hypothesis that competition between ipsi- and contralateral projection neurons is the mechanism that eliminates much of the transient contralateral projecting nigrostriatal pathway.

409.2
ULTRASTRUCTURAL CHANGES IN CAUDATE NEUROFILAMENT QUINOLINIC ACID LESIONS. J.L. Rabut and R. Schlag, Department of Neurobiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

In order to determine the anatomical basis of excitotoxicity in the caudate nucleus as a model of Huntington's disease (HD), the effects of injections of quinolinic acid (20µg in 0.6µl) into adult rat striatum were investigated at the light and electron microscopic level at 2, 4, 7 and 10 weeks post lesion. Neuronal and synaptic density were examined at each time point in four regions: 1) lesion (intact tissue exhibiting profound neuronal loss), 2) proximal to the lesion (P) at 2 and 7 weeks, 3) distal to the lesion (D) at 2 and 7 weeks, and 4) an unlesioned control side.

Results indicated that neuronal density was markedly reduced in the lesion zone at all stages, especially at 10 weeks. Relative to control, synaptic density was greatly reduced. No significant changes were observed in the proximal 2 and 7 weeks, 3) distal to the lesion (D) at 2 and 7 weeks, and 4) an unlesioned control side.

Neuronal vs Synaptic Density

<table>
<thead>
<tr>
<th>Region</th>
<th>Neuronal Density</th>
<th>Synaptic Density</th>
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<tbody>
<tr>
<td>L (lesion)</td>
<td>30%* 1 2 7 30</td>
<td>60%* 1 2 7 30</td>
</tr>
<tr>
<td>P (proximal)</td>
<td>90%</td>
<td>75%</td>
</tr>
<tr>
<td>D (distal)</td>
<td>60%</td>
<td>75%</td>
</tr>
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</table>

The lesion zone contained more neurons and synaptic terminals despite the severe loss of neuronal integrity in the surrounding area. Results suggest that excitotoxic lesions cause both depolarization and regenerative changes in caudate neurons and may parallel findings made in this laboratory in oligoparachute caudate lesions in adult brain. Supported by NS 25783 to R and K 22670 to HK.

This study examines the effects of specific striatal lesions on the release of GABA, glycine, and dopamine in lateral thalamic nuclei. The authors performed unilateral lesions in the putamen (PD) and nucleus accumbens (NA) of rats and recorded extracellularly from the lateral geniculate nucleus (LGN) and the mediodorsal nucleus of the thalamus (MD). The results showed that lesions in the PD increased GABA and glycine release, while lesions in the NA increased dopamine release. These findings suggest that the basal ganglia modulate the activity of the thalamus through the release of inhibitory and excitatory neurotransmitters.


In this study, the authors investigated the role of cerebellar Purkinje cells in MPTP-induced Parkinsonism. They recorded extracellularly from the cerebellum of MPTP-treated and control rats and found that Purkinje cells exhibited a decreased response to thalamocortical inputs, which was similar to the effects seen in Parkinson's disease patients. These results suggest that Purkinje cells may play a modulatory role in the thalamocortical pathway and contribute to the motor symptoms of Parkinson's disease.

409.5 STRIATAL Dopamine AND THE INTERFACE BETWEEN ORIENTING AND INGESTIVE FUNCTIONS. S. Hall and J. Schallert (SPON: E. Biggers). Dept. of Psychology and Institute for Neurological Science, University of Texas at Austin, Austin, TX 78712.

Recent experiments have implicated forebrain catecholaminergic projections in a unique switching mechanism that enables sensory orientation to occur during ongoing feeding behavior. Sensory-related cells identified in the striatum (Schneider et al., Neuphysiol., 1985) may serve as part of a system that redirects attention from ingestive to non-ingestive behavior and toward external stimulation (Schallert and Hall, Behav. Brain Res., 1989; Schallert and Hall, Brain Res. Bull., in press). In the present study, 6-hydroxydopamine (6-OHDA), which selectively destroys dopaminergic neurons when followed by a noradrenergic uptake inhibitor or ketamine, was infused directly and unilaterally into the ventrobasal nucleus of the thalamus and the sensorimotor cortex. Dopaminergic neurons in the substantia nigra and ventral tegmental area were lesioned, but not markedly affected by 6-OHDA. These results suggest that the striatum modulates the movements induced by 6-OHDA and are consistent with the initiation site for its action.

409.6 BOTH NITRIC OXIDE AND MUSCARINIC RECEPTORS MEDIATE THE EXCITATORY ACTIONS OF N-METHYL-D-ASPARTATE IN THE ASCENDING A9 AND A10 NEURONS. H. Mock, P. Calabresi, and R. A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from mesencephalic dopaminergic rat midbrain neurons in a slice preparation. Acetylcholine, applied by superfusion or by pressure ejection, increased the excitability of the neurons. The response consisted of a large transient component of rapid onset and a slow, more sustained component, both of which were unaffected by tetrodotoxin. The fast component increased in amplitude on hyperpolarization, was accompanied by an increase in membrane conductance, and was blocked by hexamethonium (100 µM) and cross-desensitized to nicotine (100 µM).

The slow component was mimicked by muscarine (1-30 µM) and blocked by scopolamine (3 µM). Muscarinic depolarizations and inward currents in the range -45 to -80 mV were more substantially associated with a conductance decrease. Muscarinic depolarizations and inward currents persisted at potentials negative to the K+ equilibrium potential (extracellular K+ concentration 2.5 to 20.5 mM). Dose-response curves to muscarine were shifted to the right by pirenzepine (30-300 µM), implicating an M1 type receptor in this effect.

Little is known regarding the detailed topography of the nigrostriatal system in the dog. In the present study, the autoradiographic method was systemically used in order to trace these projections. A series of bilateral injections of tritiated amino acids were made into the rostral, caudal, lateral and medial parts of the substantia nigra (SN) in anesthetized dogs. After survival times of 3-7 days, the animals were perfused with formalin-saline and the brains processed for standard autoradiography. Nigrostriatal projections terminated in a widespread patch-like pattern in the ipsilateral striatum (St). A topographical relationship emerged whereby obliquely oriented longitudinal slabs of the St received a rostro-caudal and mediolateral inverted nigral input. The lateral portion of SN projects to the ventromedial part of the St while the medial part of SN projects to the dorsolateral St. Injections confined to the rostral half of SN primarily labeled the caudal part of St. These results suggest that the topographical organization of the canine nigrostriatal system may be more complex than that previously described in other species. (Supported by N.I.H. Grants NS14991 and NS18551 and B.R.S.C. funds to the Colleges of Human Medicine and Veterinary Medicine, M.S.U.)


Previous studies have indicated that the substantia nigra, pars reticulata (SNr) is involved in the control of certain aspects of motor behavior and may mediate these effects through projections to the substantia nigra pars compacta (SNc). To determine the light and electron microscopic study utilizes the anterograde transport of lectin Phascolus vulgaris (PHA-L) to delineate the origin, distribution and mode of termination of nigral fibers in the PPN. Small and large injections of PHA-L in the PPN resulted in labeling within the SNR resulting in similar distribution of the terminal field in the PPN. Both subdivisions, the subnucleus compactus (PPNC) and disalpatautum (PPND) appeared to receive the nigral afferents. However, the overall projections to the PPN were more prominent than that to the PPN. A particularly dense plexus was seen in the medial PPN in close approximation to the superior cerebellar peduncle. Although the terminal varicosities were often seen on the somas of PPN neurons, the electron microscopic examination revealed mostly termination on the dendrites. The observations suggest that the nigral afferents are likely to originate from the entire SNR and do not exhibit any topographical organization. They terminate throughout the PPN and may impinge on dendrites of various populations of the PPN projection neurons. (Supported by N.I.H. Grant NS25744).


Previous studies have indicated that the basal ganglia may control movement through a pathway involving the nucleus pedunculopontinus (PPN). Since there exists only a meager projection from the PPN to the spinal cord, it is presumed that the PPN is linked to the spinal motor system through the reticular formation (RF) of the brainstem. This study utilizes the anterograde transport of lectin Phascolus vulgaris (PHA-L) to delineate the distribution of PPN fibers in the pontine and medullary RF of the rat. Following iontophoretic injections of PHA-L involving only the PPN, a significant proportion of labeled axons course caudally through the pontine and medullary RF. Within the nuclei reticularis pontis oralis and caudalis some of the axons collateralize to form a dense axonal plexus within these nuclei. Many labeled axons terminate within the medullary RF, particularly in the ventromedial portions of the nuclei reticularis gigantocellularis and its subdivisions (Cv, PGI), paramedian reticular nucleus and the ventral reticular nucleus of the medulla. The results provide definite evidence of a substantial PPN projection to the pontine and medullary reticular nuclei containing reticulospinal neurons. They further support the notion that the PPN may serve as a relay between the basal ganglia and lower motor system. (Supported by N.I.H. Grant NS25744).


As part of our research to determine the pattern of innervation of extrastriatal afferents to the substantia nigra, we injected the anterograde tracer PHA-L i.t. in the anterior hypothalamic area (AHA) and the amygdalo medial and lateral nuclei of the rabbit. After survival times of 14 days, animals were perfused transcardially, the brains were removed and 30 mm thick sections were taken. The presence of PHA-L was determined immunocytochemically using the avidin-biotin system with diaminobenzidine as the chromogen. Injection sites that encompassed most of the central nucleus as well as discrete injections of the medial subregion resulted in immunocytochemically labeled axons and boutons in the medial aspect of the substantia nigra pars compacta. Injection sites that involved either the medial or lateral and lateral subdivisions were confined to the ventral part of the nucleus produced some labeling in the lateral aspect of the pars compacta and few if any labeled processes in substantia nigra pars lateralis. In one case, extensive labeling of axons and boutons was seen in both parts lateralis and the lateral division of pars compacta when the injection site was in the most dorsal part of the central nucleus. Immunoreactivity was not seen in the substantia nigra pars reticulata after anterograde injections. The results suggest that central nucleus of the amygdala projections to the substantia nigra are more extensive than previously reported. Furthermore, subregions of the central nucleus project to different parts of the substantia nigra suggesting an heterogeneous organization of the pathway. Supported by NNS 86-07454 and the Dysstonia Medical Research Foundation.

Immunohistochemical studies with adult onset Huntington’s disease (HD) have revealed that enkephalin (ENK)-positive fibers in the external pallidum, and substance P (SP)-positive fibers in the substantia nigra were decreased at earlier stages of the disease than SP-positive fibers innervating the internal pallidum (Albin et al., Neurosci. Abs. ’87, 1961). In order to determine whether a similar pattern of loss occurs during normal aging in the rat, we analyzed met-ENK- and SP-like immunoreactivity in sections of brain from Fisher 344 rats aged 4, 16 and 24 months. In a single experiment, immunohistochemistry still revealed a dense plexus of ENK-positive fibers in the globus pallidus, and of SP-positive fibers in both the substantia nigra and the entopeduncular nucleus in 24 month old rats. ENK and SP immunostaining was also observed at all ages examined using a 125I-labeled secondary antibody and film autoradiography. Quantification of optical densities in the films using a UNIPHASA image analysis system revealed a slight decrease in SP-like immunoreactivity in both substantia nigra and entopeduncular nucleus in the aged rats. The results show that the pattern of alteration of striatal efferent neurons during normal aging in Fisher 344 rats does not parallel that seen in patients with HD. This further supports the hypothesis that the differential alteration of striatal efferent neurons observed during HD is related to a specific effect of the deficient gene. Supported by BNS 86-16841.

409.17 STUDIES ON Dopameric projections ARIgInG In the Dorsal Raphe. J. R. Stratford and B. Wirtschafter. Dept. Psych., Univ. of Illinios at Chicago, IL 60680.

While the serotonin efferents of the dorsal raphe (DR) have been extensively investigated, little is known about the terminal sites of non-serotonergic efferents. In the following study, adult male rats received 0.1 μl injections of the fluorescent retrograde tracer Fast Blue bilaterally into the substantia nigra (SN), the striatum (CPU) or the nucleus accumbens (Acb). Following a 5 day survival period, the brains were removed and processed for the immunohistochemical detection of dopamine hydroxylase using a rhodamine-labeled secondary antibody. In each of the brains a population of tyrosine hydroxylase-immuno­reactive (THI) neurons, apically continuous with those in the caudal linear nucleus, was observed in the rostral aspect of the DR. Although a large number of retrogradely labeled cells projecting to the CPUs or the SN were observed intermingled with the THI neurons, very few of the THI neurons were double-labeled following CPU injections and none following SN injections. In contrast, approximately 10% of the THI cells in the DR were double-labeled following injections confined to the Acb. The double-labeled cells appeared to be distributed uniformly throughout the THI cell population.

In conclusion, the projection pattern of THI cells in the DR appears to differ significantly from that of the serotonergic population and seems to resemble that of THI cells in the ventral tegmental area. (Supported by NS12150)

409.19 PHAL STUDIES OF THE DIFFERENTIATED CONNECTIONS OF RAT GLOBUS PALLIDUS. Wm. A. Staines, Department of Anatomy, University of Ottawa, Ottawa, ONT. K1N 6N9.

The projections of the globus pallidus of the rat were studied using anterograde tracing with Phaeus vulgaris Leucoagglutinin (PHAL) in combination with retrograde fluorochrome tracing methods and immunohistochemistry for neurotransmitter markers. Anterogradely injected neurons were found in the caudate-putamen, reticular nucleus of the thalamus, entopeduncular nucleus, subthalamic nucleus and the substantia nigra. A single (fibrous) axon was found within all of these sites, consisting of large, regularly spaced varicosities en passant. A few fibers with similar morphology were found within reticular regions of the frontal cortex as well. Pallidal efferents to the substantia nigra showed a striking, perisomatic clustering about the cell body and primary dendrites of GABAergic output neurons. A similar but less dramatic perisomatic pattern of termination was seen around somatostatin-containing neurons of the caudate-putamen. (Supported by a grant from the NRC).

409.20 DISTRIBUTION OF CHOLINERGIC PERIKARYA WITH RESPECT TO HETEROMORPHIC SUBSTANCE P STAINING IN THE CORPUS CALLOSUM. NUCLEUS OF THE CAT. M. Martone*, D. M. Armstrong, P. M. Grove. Univ. California San Diego, CA 92093.

Both substance P (SP) and choline acetyltransferase (ChAT) are known to be distributed heterogeneously in the mammalian striatum. In the present study, we examined the relationship between cholinergic perikarya and SP immunoreactivity in the caudate nucleus of the cat. Adult cats were injected intracranially with PHAL using a double-label immunocytochemical protocol. Fifty micron sections were cut throughout the extent of the caudate nucleus and labeled sequentially for ChAT (antibody provided by L. Herdman), and SP (Sera Labs). To distinguish the two labels at the light microscopic level, nickel chloride (NicCl) was added as an intensifier to the dianinobenzidine (DAB) reaction for the ChAT antibody. This resulted in a purple reaction product that was clearly distinguishable from the light brown reaction product of the intensified DAB reaction used to localize SP. Preliminary observations suggest that ChAT-positive perikarya are more numerous in striatal areas that contain SP densely, and are more concentrated in the SP-rich matrix. Supported by ONR 000-14-85-K-0699 and NIDA 00079 to PMG and NIA AGO 3544 to DMA.
409.21


The striatongigant pathway contains several neurotransmitters, two of which are considered to represent excitatory innervation of nigrostriatal dopaminergic neurons: substance P and neurokinin A. Local injection of substance P (0.00007-7.0 nmol/0.2 µL) or neurokinin A (0.009-9.0 nmol/0.2 µL) into the substantia nigra, pars reticulata (SNR) of halothane anesthetized male Sprague-Dawley rats produced long lasting increases in ipsilateral striatal DA as measured by in vivo microdialysis. Intranigral injections were repeated in animals with ibotenic acid lesions (5.0 µg/0.5 µL) impairing non-dopamnergic neurons of the SNR (preliminary studies show only minor damage to the tyrosine hydroxylase positive dendrites in area of lesion). In the lesioned animals substance P (0.07 nmol/0.2 µL) injected into SNR significantly impaired acquisition as measured by receptor activation. 

**401.1**

GALANIN ANTAGONIZES ACETYLCOLINE ON A MEMORY TASK IN BASAL GANGLIA AND THALAMUS: MOTOR SYSTEMS V

G. Schulteis & J. L. Martinez. Jr, & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720

Galanin coexists with acetylcholine in nucleus basalis magnocellularis (NBM) and medial septal (MSA) neurons projecting to the cerebral cortex and hippocampus in primates (Melander and Staines, Neurosci Lett, 1986; Walker et al., Neurosci, 1987), and in MSA neurons projecting to the ventral hippocampus in rats (Melander et al., Brain Res 1985), where galanin inhibits the release of acetylcholine (Fitzone, PNAS, 1987). To investigate the possible behavioral role of galanin in memory processes thought to be mediated by these pathways, male Sprague-Dawley rats were lesioned with ibotenic acid at five sites in the NBM-MSA (Helpert et al., Brain Res 1985), and trained on a delayed alternation task for a food reward. Acetylcholine, 7.5 or 10 µg i.v.t., or 1 µg into the ventral hippocampus, significantly reversed the performance deficit in the lesioned rats. Galanin, 100-500 ng i.v.t., or 200 ng into the ventral hippocampus, attenuated the ability of acetylcholine, 10 µg, to restore t-maze performance in sham-lesioned control rats. These data suggest that galanin may inhibit cholinergic function in brain pathways relevant to Alzheimer’s disease.

**401.2**

BETA-ENDORPHIN ADMINISTRATION IMPAIRS ACQUISITION IN THE CHICK: FIRST REPORT OF OPIOID EFFECTS ON MEMORY FORMATION IN AN AUTOMATED SHELF-JUMP TASK IN RATS. S. B. Weinberger.

Weinberger et al., in press). To determine the post-training susceptibility gradient for beta-endorphin, groups of chicks were given bilateral injections into the Mtv of either saline or beta-endorphin (1.0 nmole per hemisphere) 5 min before training, or at various times after training. The chicks were trained 24 hr after training. The results indicate that beta-endorphin is amnestic when given 5 min before training, but is not amnestic when given at any time after training. These results suggest that this opioid acts on an early stage of memory formation.

To examine the time course of amnesia development, chicks were given bilateral injections into the Mtv of either saline or beta-endorphin (1.0 nmole per hemisphere) 5 min before training, and groups of chicks were tested at various times after training. The results indicate that beta-endorphin produces amnesia that is present by 10 sec after training; this amnesia is permanent. These results indicate that in chicks, as in rodents, pre-training injection of beta-endorphin produces an impairment in acquisition.

**401.3**

FURTHER STUDIES ON THE ROLE OF OPIOID DELTA RECEPTORS IN THE EFFECTS OF LEUDENKEPHALIN ON ACTIVE AVOIDANCE CONDITIONING TO 80° C IN MALE SWISS-WEBSTER MICE. N. A. Schon*, J. L. Martinez, J. Jr, & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720

To determine the post-training susceptibility gradient for beta-endorphin, groups of chicks were given bilateral injections into the Mtv of either saline or beta-endorphin (1.0 nmole per hemisphere) 5 min before training, or at various times after training. The chicks were trained 24 hr after training. The results indicate that beta-endorphin is amnestic when given 5 min before training, but is not amnestic when given at any time after training. These results suggest that this opioid acts on an early stage of memory formation.

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**401.4**

LEUDENKEPHALIN AND ITS DELTA-SELECTIVE ANALOGS, D-PEN2-6-PHENILENKEPHALIN (DPDPE), IMPAIR AVOIDANCE CONDITIONING IN AN AUTOMATED SHELF-JUMP TASK IN RATS. S.B. Weinberger, C. Schon*, J.L. Martinez, J. Jr, & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720

We previously reported (Neurosci Abstr 135:485, 1987) that selective stimulation of opioid delta receptors with D-Pen2,6-DPhe (DPDPE), a delta opioid receptor-selective analog of LE, impairs acquisition of active avoidance, as does LE itself. In the present study, we determined whether the delta opioid receptor agonist 5-methyl-leucine enkephalin (DPDPE), a delta opioid receptor-selective analog of LE, also impairs acquisition in mice. (Supported by NASA #DAO2195.)
QUATERNARY FORMS OF NALOXONE AND NALTREXONE ENHANCE ACQUISITION OF ONE-WAY ACTIVE AVOIDANCE CONDITIONING IN RATS. N. Pietrusiak*, L. Rumennik*, G. P. Vincent and J. Sepinwall (SPON: A. Davidson). Neurobiol. Aging, 5:621-627, 1984. Pre-training ip injections of naltrexone (NEM) enhanced acquisition of one-way active avoidance conditioning (psychopharmacol., 87:410, 1985). However, naltrexone methyl bromide (NB) failed to alter passive avoidance retention when administered pre-training (Behav. Neural Biol., 44:434, 1985). This apparent discrepancy may reflect: 1) different administration; 2) differences in conditioning paradigm employed; or 3) differences in pharmacological properties of NEM and NB. The present study therefore compared the effects of pre-training injections of NEM and NB on one-way active avoidance acquisition.

Male Swiss Webster mice were given [leu]enkephalin, NEM, N-Me, or saline pre-training to two minutes prior to trials (Neurosci., 13:845, 1987). [leu]enkephalin (100 ng/kg) impaired acquisition of the avoidance response. NEM (0.1 & 0.2 mg/kg) enhanced acquisition, but 0.1 mg/kg was ineffective. These results suggest that both quaternary opioid agonists exert similar modulatory effects on one-way active avoidance conditioning when administered prior to training. The effects of post-training injections of NEM and NB on retention of active avoidance conditioning are currently being investigated. (Supported by PHS grant DA 04195).

EFFECTS OF POSTMATERNAL TESTOSTERONE PROPERGATE ADMINISTRATION ON RADIAL ARM MAZE PERFORMANCE IN RATS. R. L. Root. Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY. 82071.

This study examined the hypothesis that sex differences in spatial ability are due in part to differences in left-right hormone environments during brain development. Male and female rats were tested with either 75 or 150 μg testosterone propionate (TP) or an oil vehicle on days 4 and 6 after birth. At 90 days of age, the rats were trained to 8% criterion level of performance on a radial arm maze. The rats were tested once a day for 10 successive days. Rats were then tested for 24 trials on a task in which 8 of the 3 arms were baited. Accuracy and pattern of arms entered were recorded. Female rats treated with TP reached the criterion level of performance in significantly fewer trials than did control females, while the opposite pattern was observed for males. A dose related effect was observed for females, with the higher dose producing better performance. Control rats entered significantly more adjacent arms in sequence than did rats treated with TP. Males entered fewer adjacent arms in sequence than did females. No overall sex differences for accuracy were observed once criterion levels of performance were reached. These findings support the hypothesis that steroid hormones have an organizational effect on the developing brain that subsequently influences cognitive functioning.


CCK-8 has been reported to alter the acquisition or retention of learned responses in animals although the nature of its effect, improvement or impairment, has also significantly decreased the latency to find the hidden platform in a Morris water maze; the duration of the curve. In C57BL/10 mice, CCK-8 (0.03-0.3 mg/kg s.c.) also significantly decreased the latency to find the hidden platform in a Morris water maze; the duration of the curve. In C57BL/10 mice, CCK-8 (0.03-0.3 mg/kg s.c.) also significantly decreased the latency to find the hidden platform in a Morris water maze; the duration of the curve. In C57BL/10 mice, CCK-8 (0.03-0.3 mg/kg s.c.) also significantly decreased the latency to find the hidden platform in a Morris water maze; the duration of the curve.
3.2, or 5.6 ug/kg, s.c. 1 hr before test) had no effect, whereas SCOP alone (17.8 ug/kg, i.m. 20 min before test) produced a significant memory loss. When both compounds were given, AVP (1.78 and 3.2 ug/kg) significantly attenuated the SCOP-induced memory impairment, even though scores did not return to control levels. Although the mechanism for this attenuation is unknown, the results encourage further study of the possible therapeutic effects of AVP.

**EPILEPSY: PEPTIDES**

**411.1**

**DIFFERENTIAL CHANGES OF THE LEVELS OF PROENKEPHALIN AND PREPRODYRPHIN M RNAs IN RAT BRAIN DURING THE DEVELOPMENT OF DEEP PREPYRIFORM CORTEX KINDLING.**

P. H. Lee, C. W. Hong, Lab. of Molecular and Integrative Neuroscience, NIEHS, Research Triangle Park, NC 27709.

We have demonstrated by repeated subconvulsive electrical stimulations of the deep prepyriform cortex (DPC) led to an early increase of Met-enkephalin but not dynorphin in the hippocampus before the development of generalized convulsions. This study investigates whether such change is due to an increase in preprodynorphin (DYN) mRNA during the development of DPC kindling. Rats were stimulated every hour until twice consecutive Stage 2 (S2) or Stage 5 (S5) occurred and were sacrificed either 3 or 24 h later. At 24 h pEKR mRNA in the entorhinal cortex and striatum was significantly increased. pEKR mRNA was also increased in the hippocampus, frontal cortex, and entorhinal cortex 5 min after S5, whereas 24 h after S5, significant increases were found in the frontal cortex and entorhinal cortex but not in the hippocampus. Hippocampal preprodynorphin (pDYN) mRNA was significantly reduced 24 h after S5 but not at the other time periods studied. These data demonstrate that pEKR and pDYN gene expressions were differentially regulated during the development of DPC kindling. The early increase in pEKR mRNA content suggests that enkephalinergic system may play a role in kindling development.

**411.2**

**ELECTROCONVULSIVE SHOCK ALTERS CONTENT OF mRNA CODING FOR PREPRODYRPHIN AND PREPROENKEPHALIN IN DIFFERENT RAT BRAIN REGIONS.**

C. W. Lee, P. H. Lee, J. Douglass*, and J. S. Hong, Lab. of Molecular and Integrative Neuroscience, NIEHS, RTP, NC 27709 and Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR.

The effects of single or repeated electroconvulsive shocks (ECS) on abundance of dynorphin and enkephalin mRNA in rat brain were investigated. Rats were subjected to 1, 3 or 6 daily ECS and sacrificed 24 h later. The amount of mRNA coding for dynorphin (DYN) and enkephalin (EK) mRNA were measured in several brain regions using RNA blot analysis. ECS led to a progressive decrease in hippocampal DYN mRNA with maximum reduction of 51% after 6 consecutive ECS. A consistent but lesser decrease in DYN mRNA level was observed in the entorhinal cortex 24 h after 1, 3, or 6 daily ECS. In contrast, DYN mRNA level showed significant elevation (by 83%) in striatum, and a modest increase (by 33%) in hypothalamus after a single ECS. In contrast, DYN gene expression gradually declined after 3 or 6 daily shocks. EK mRNA was found to increase in entorhinal cortex (57%) and hypothalamus (75%), whereas only a small decrease was observed in hippocampus and no change in striatum after 6 daily ECS. These data suggest that repeated ECS enhances the biosynthesis of enkephalin but inhibits that of dynorphin in the entorhinal cortex-hippocampal region.

**411.3**

**LOCALIZATION AND QUANTIFICATION OF VIP, SS, AND GABA IMMUNOREACTIVITY IN SEIZURE-INDUCED KINDLING.**


The relationship between transcription and translation of a number of neuropeptides in the rat brain during the development of temporal lobe epilepsy, experiences tonic-clonic seizures of hippocampal origin, and during postictal stimulation. No previous work has documented an anatomical correlation for the seizure network. For EL mice were killed by gentle stunning at 30, 30, and 44 days after Stage 5 seizures. At 57 days after Stage 5 seizures, all EL mice had experienced at least 10 seizures. Presence of VIP, SS, and GABA was determined using PAP immunohistochemistry. We observed elevated VIP, SS, and GABA levels in specific hippocampal and neocortical subdivisions of EL mice when compared to controls. These findings suggest that different stages of kindling development may be associated with different changes in neuropeptide expression. These studies demonstrate that the VIP, SS, and GABA systems play important roles in the development and maintenance of temporal lobe epilepsy. Further studies are needed to determine the mechanisms by which these neuropeptides are affected during kindling development.

**411.4**

**THE TIME COURSE OF CHEMICAL CHANGES IN THE KINDLED RAT BRAIN.**

N.S. Nag, NINDS, Bethesda, MD.

The evolution of changes in amino acids, neuropeptides, and catecholamines were studied in the cortex (CX) and hippocampus (H) of kindled rats at stages 1, II, III, IV and V of seizures. The levels of the amino acids glutamate, aspartate, GABA and glycine were unchanged throughout the evolution of seizures in CX and H. Somatostatin increased at stage I in CX but not H and continued to increase at a maximal level at stage V in the CX. The changes in somatostatin in the H were not as marked as those observed in the cortex. The levels of substance P, neuropeptide Y, dynorphin and VIP were not altered at any of the stages in CX or H. The levels of enkephalin increased in the H at stages IV and V, but were unchanged at the earlier seizure stages. Enkephalin levels were not markedly altered in the CX. The levels of catecholamines were not altered at any of the stages in CX or H. The number of MEA receptors was slightly increased at stage II in the H and was unchanged in the CX. The levels of dopamine and norepinephrine were significantly increased in CX and H. The dynamic interactions between these changes are currently being investigated using slice techniques.
**411.5**

**LAMINAR DISTRIBUTION OF PEPTIDES, CHOLINE ACETYLTRANSFERASE AND CYCLIC GMP IN THE HUMAN EPILEPTIC CORTEX AND HIPPOCAMPUS.** K. Waun*, A.R. Wyler* and N.S. Nadi* NINCOMS, Bethesda, Md. (SPON: E.T. Hambrecht)

We measured the distribution of choline acetyltransferase (CAT), neuropeptide Y (NPY), somatostatin (ST), glutamate (glu), aspartate (asp) and QNB and MMDA receptors in the cortex and hippocampus of the kindled rat. The changes in cyclic AMP content were not statistically different between kindled and sham-operated rats. The increase in cyclic GMP in the kindled cortex and hippocampus slices was significantly higher when compared to the sham operated cortex and hippocampus. These preliminary experiments suggest that the control of cyclic GMP pool is altered in the cortex and the hippocampus of the kindled rat brain. Such alterations in second messenger control may represent a step in explaining the molecular mechanisms of the changes underlying the kindling phenomenon. Studies are currently underway to determine the time course of these changes as well as the effect of the above compounds on the phosphatidylinositol class of second messengers.

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**411.6**

**CONTENT OF NEUROTENSIN, VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ IN THE HUMAN EPILEPTIC CORTEX AND THE HIPPOCAMPUS.** K. Waun*, A.R. Wyler* and N.S. Nadi* NINCOMS, Bethesda, Md. (SPON: E.T. Hambrecht)

The levels of the neuropeptides neurotensin (NT), vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK) were determined in the spiking and non spiking regions of the temporal lobes surgically removed from 10 intractable epileptics. CCK was lower in the spiking cortex (12.5±2.8 pg/mg prot.) vs the non spiking cortex (22.9±1.7 pg/mg prot. p<0.01). NT was higher in the spiking cortex when compared to the non spiking cortex, 0.82±0.2 pg/mg prot. and 0.59±0.2 pg/mg prot. respectively (p<0.01). VIP was also lower in the spiking vs non spiking cortex 2.14±0.4 pg/mg prot. vs 6.2±1.0 pg/mg protein respectively (p<0.01). The levels of NT in six epileptic hippocampi were 0.75±0.03 pg/mg prot.. The levels of VIP and CCK were 25.5±8.2 and 11.9±4.0 pg/mg prot. respectively. No autopsied hippocampus was available for comparison. The role for most neuropeptides in the central nervous system is unclear. NT is known to colocalize with dopamine (DA) neurons and also be excitatory. Our previous studies have shown an increase in DA in the spiking cortex and it is possible that NT or DA may be colocalized. NT may also regulate the excitability of the region. VIP has been implicated in control of circulation and the alterations observed suggest a disorder in regional circulation.

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**411.7**

**SECOND MESSER SYSYSTEMS IN THE KINDLED CORTEX AND HIPPOCAMPUS.** N. Mai* and N.S. Nadi* NINCOMS, Bethesda, Md. (SPON: W.H. Theodore)

We have investigated the effects of norepinephrine (NE), increased Kt, N methyl aspartate (NMA) with respect to cyclic AMP and cyclic GMP regulation in slices prepared from the cortex and the hippocampus of the stage V kindled rat. Increased Kt caused a significantly larger increase in the kindled rat hippocampus when compared to the slices from sham operated rats. The changes in cyclic AMP content were not statistically different between kindled and sham operated rat brain slices. Cyclic AMP was increased in response to exposure of the cortex and the hippocampus slices to norepinephrine. These changes were not statistically different between sham operated animals. The changes in cyclic GMP content in the cortex and hippocampus slices was significantly higher when compared to the living operated cortex and hippocampus. These preliminary experiments suggest that the control of cyclic GMP pool is altered in the cortex and the hippocampus of the kindled rat brain. Such alterations in second messenger control may represent a step in explaining the molecular mechanisms of the changes underlying the kindling phenomenon. Studies are currently underway to determine the time course of these changes as well as the effect of the above compounds on the phosphatidylinositol class of second messengers.

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**411.8**


Previous studies in our laboratory have discovered a number of alterations in neuropeptides, catecholamines, amino acids, receptors and enzymes in the human epileptic focus. With results from 35 patients we have analyzed the data with respect to cross correlations between these findings as well as the relationship to surgical outcome. The data addressed in these analyses were from a variety of samples, all of which were from intractable epileptics. The data were analyzed to determine if there was any correlation between surgical outcome and the neuropeptide alterations. The cross correlations revealed a statistically significant correlation between somatostatin and VIP in the temporal lobe (r=0.62 p<0.01). A similar degree of correlation was observed between VIP and POMC (r=0.79 p<0.01). No other significant correlations were observed in this study. There was no significant correlation between the transmitter and neuropeptide alterations. The data reported here did not correlate with surgical outcome. Study of the effects of these neuropeptides on the hippocampus and the cortex and the relationship to surgical outcome is ongoing. The role for most neuropeptides in the central nervous system is unclear. NT is known to colocalize with dopamine (DA) neurons and also be excitatory. Our previous studies have shown an increase in DA in the spiking cortex and it is possible that NT or DA may be colocalized. NT may also regulate the excitability of the region. VIP has been implicated in control of circulation and the alterations observed suggest a disorder in regional circulation.

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**411.9**


The levels of serotonin (5HT), dopamine (DA), and their metabolites, SHIM and HVA were measured in surgically resected and surgically operated rats. The changes in cyclic AMP content were not statistically different between kindled and sham operated rat brain slices. Cyclic AMP was increased in response to exposure of the cortex and the hippocampus slices to norepinephrine. These changes were not statistically different between sham operated animals. The changes in cyclic GMP content in the cortex and hippocampus slices was significantly higher when compared to the sham operated cortex and hippocampus. These preliminary experiments suggest that the control of cyclic GMP pool is altered in the cortex and the hippocampus of the kindled rat brain. Such alterations in second messenger control may represent a step in explaining the molecular mechanisms of the changes underlying the kindling phenomenon. Studies are currently underway to determine the time course of these changes as well as the effect of the above compounds on the phosphatidylinositol class of second messengers.

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**411.10**

**HIPPOCAMPAL VOLUMETRIC NEURAL DENSITY IN TEMPORAL LOBE EPILEPSY.** J.A. Kim, P.O. Gurumaran*, M.Y. Shen*, C. Dobert*, S.S. Spencer, D.D. Spencer, Dept. of Surgery (neuropathology, neurosurgery) and Neurology, Yale Univ. School of Medicine, New Haven, CT 06510

Hippocampal sclerosis, shown in a majority of temporal lobe epilepsy cases, is histologically composed of neuronal loss and gliosis, and its exact pathogenesis is controversial. The relationship between the degree of neuronal loss and clinical variables, volumetric neuronal density was measured in the hippocampal fields from the mid or mid-posterior level of surgically removed hippocampal intractable epileptic cases. Eleven age matched autopsy cases without any previous history of seizure or other neurological illness were included in these analyses. The data were generated with Abercrombie formula, and expressed as mean neuronal number per mm3. Pearson's correlation was calculated for each variable and was statistically significant (r=0.62 p<0.01). There was a significant correlation between neuronal numbers and blood flow to the temporal lobes. The lack of correlation with outcome may reflect the fact that surgery only removes a portion of the tissue required to stop the seizures, but that it is not always necessary to remove all the tissue to have good outcome. The data will be discussed with respect to animal models and a chemical identification of the spiking region.
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Epileptiform field potentials were recorded from CA1 and the parameters of the or number of population spikes in either physiological (0.36-36 nM) or epileptiform activity.

Does not modify epileptiform activity in the hippocampus at either physiological or pharmacological doses. Investigations of the mechanism of action of estrone-3-sulfate produced epileptiform activity. The effects of estrone did not occur in the granule cell and molecular layers, sometimes extending through the inner third of the molecular layer. Reaction product was also concentrated around the limited number of neurons remaining in the hilus and CA3 field.

These findings suggest that morphological reorganization of mossy fibers has occurred in humans with temporal lobe epilepsy, as demonstrated in animal models of seizure disorders (Nadler, J.V. et al., 1980), and indicate that the reorganized fibers contain at least one of the neuroactive substances normally present in mossy fibers. Supported by VA Medical Research Funds and NIH Grant NS 21908.

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Corticotropic-releasing hormone (CRH) is a stress-related peptide which causes seizures of late onset [1-5 hour latency following intracerebroventricular administration] that involve limbic structures. In vitro, CRH causes the release of beta-endorphin, a peptide which produces kindled seizures following intra-amygdaloid or hippocampal administration (Cain & Corcoran, 1984). On the other hand, endogenous opiates released during seizures may be related to decreases in seizure susceptibility. To evaluate the role of opiate peptides in CRH-induced seizures we investigated naltrexone, an opiate antagonist (10 mg/kg, i.p.) one-half hour prior to, and 4 hours after (5 mg/kg) CRH (100 µg, i.c.v.) on 2 consecutive days. The overall effect of naltrexone was to potentiate CRH-induced seizures. Although the number of animals experiencing seizures did not differ on day 1, the number of seizures per animal tended to be greater in the naltrexone group than in the controls (X̅ = 5.71 ± 1.52 vs. 2.37 ± 0.53); the latency tended to be shorter in the naltrexone group (100 mg/kg CRH injected i.c.v. on day 1; 10 mg/kg CRH injected s.c. on day 2). On day 4, naltrexone-treated rats exhibited seizures (69% compared to 31% controls), although the overall number of seizures on this day did not differ between groups. Thus, opiate release is unlikely to be the mechanism responsible for CRH-induced seizures. The trend for an overall proconvulsant effect of naltrexone on several measures may be related to a blockade of post-ictal opiate effects which in other seizure models are inhibitory.

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DIETHYLTHIOICARbamate AND DITHIOZINE AUGMENT THE TOXICITY OF KAINIC ACID. C.L. Mitchell, M.I. Barnes 1, and L.A. Grimes. Lab. of Molecular and Integrative Neuroscience, NIEHS/NIH, Research Triangle Park, NC 27709 and Toxicology Curriculum, UNC-Chapel Hill, NC 27599.

Grimes et al. (J. Neurosci., 8, 1320-1326, 1988) reported that colchicine-induced lesions of the hippocampal mossy fiber pathway eliminate kainic acid (KA)-induced wet dog shakes (WDS) but do not affect the latency to onset of seizures. Since colchicine appears to function in a functional dissection of this pathway (Crawford and Connor, Orthomolec. Psych., 4, 39, 1975) we wished to determine the effects of the 2-chloroethyl esters of the diethylthiocarbamate (EDTC) and dithizone (D) on KA-induced WDS and seizure activity. Male Fischer-344 rats were injected i.p. with EDTC (100, 200, or 400 mg/kg) or D (12.5, 25, 50, or 100 mg/kg) 15 min. after KA (8 mg/kg, s.c.). EDTC and D increased the latency to onset of seizures induced by KA. Moreover, they increased the severity of seizures and frequency of death. Doses as low as 100 mg/kg EDTC and as low as 50 mg/kg D were effective. For example, 7/10 animals receiving EDTC (100 mg/kg) exhibited hind limb clonic seizures and 8/10 died following KA whereas these effects were seen in only 2/10 of the animals receiving KA plus the vehicle control. These compounds may be useful tools for investigating the role of Zn in central nervous system excitatory neurotransmission and/or convulsive phenomena.
REGIONALLY SPECIFIC EFFECTS OF DIAZEPAM ON 2-DG UPTAKE DURING STATUS EPILEPTICUS IN KINDLED RATS. B.E. Jones. Mild motor components. We examined 2-DG uptake patterns during early pfSE in rats treated after 10 min of pfSE by mild motor components. Diazepam treated rats showed a similar bilateral increase in 2-DG uptake pattern except for a selective bilateral decrease in hippocampal pyramidal cells. Thus, the inhibitory effect of diazepam on 2-DG uptake in the hippocampus and some neocortical areas was not significant factors in the sensitization process.

412.2 EXCITATION BY GABA IN MOUSE SPINAL CORD. G. Osuka and G.G. Somjen. Dept. Physiol. Duke Univ. Durham NC 27710. Last year we reported that spontaneous activity is induced in hemisected mouse spinal cord in vitro by elevated Ca2+ [p] (Neurosci. Soc. Abstr. 15:318, 1987). Similar activity was induced by high Ca2+ [p], low Mg2+[p] and low Mg2+[p] induced spontaneous discharges in both dorsal and ventral roots (DRs and VBA) which were blocked by the GABA-agonist SR 95531 and bicuculline. After clearing macroscopic events, spontaneous activity was induced in VR by the perfusion of 30 mg/kg i.v. 1,2-diamine, GDEE, or propylene glycol injected in the cord. With continued injection of 1,2-diamines, GDEE, or propylene glycol, 1,2-diamines depress spontaneous in the cord. With continued injection of 1,2-diamines, GDEE, or propylene glycol, 1,2-diamines depress spontaneous firing rates in 2 of 6 cortical neurons, but only 1 of 6 hipocampal pyramidal cells were depressed. It is concluded that U-54988H and other 1,2-diamines inhibit neuronal excitability by a non-kappa receptor mechanism.

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similar to anticonvulsant efficacy against GTC seizures, MBZB may function as a general anticonvulsant binding site. Thus, the pattern of [3H]FNZ photolabelling was observed with diazepam, phenytoin, autoradiography. Reversible, concentration-dependent displacement of [3H]FNZ photolabelling to BZBP is saturable, stereoselective and displaceable with other photoactive BZs. In this study, we report evidence for the presence or absence of non-photoactive competitor. Quantitation of [3H]FNZ binding to BZBP was performed by SDS-PAGE and autoradiography. Reversible, concentration-dependent displacement of [3H]FNZ photolabelling was observed with diazepam, phenytoin, carbachol, and disodium 4,4'-diisethylnaphthalene, which are not effective against GTC seizures, MES-induced seizures and SFA.

The high (μM) P-BZD concentrations involved suggests that the P-BZD binding site involves differences from the high (μM) affinity mitochondrial P-BZD receptor. (Supported by an O.N.H.F. Studentship to J.F.)

PERIPHERAL-TYPE BENZODIAZEPINE LIGANDS REGULATE SPECIFIC \( \beta \)-PHENTYTOIN BINDING TO RAT BRAIN WHOLE CORTEX MITOCHONDRIA. J. FRANCIS and L. SPERO, Dept. of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 3A8.

These studies were conducted to assess specific binding sites for \( \beta \)-phenytoin (\( \beta \)-DPM) in rat brain and the regulation of these sites in vitro. These sites, previously reported to be concentrated in the fraction of rat brain homogenates, were localized in the mitochondrial fraction but not in synaptosomes. Peripheral-type benzo diazepine ligands (BZs) exhibit high affinity binding to mitochondrial outer membrane, modulated specific \( \beta \)-DPM binding to mitochondrial fractions. P-BZD agonists enhanced both total and \( \beta \)-DPM binding in a dose-dependent manner. The order of BZs was flumazenil \( \alpha \)-BZB: 1.05-1.1, PHT: 1.10, 1.87, 4.9, 6.9, 10.6: 1, 8.0, 4.9, 6.9, 10.0. HindIII-cut DNA, when probed with a cDNA representing the C-terminal portion of the alpha subunit, gave the following pattern of bands (approx. sizes in kbp): DZP: 2: 1.10, 1.87, 4.9, 6.9, 10.0; C57BL/6: 1.05, 1.80, 4.9, 6.9, 10.0. HindIII-cut DNA, when probed with a cDNA representing the C-terminal portion of the alpha subunit, gave the following pattern of bands (approx. sizes in kbp): DZP: 2: 1.10, 1.87, 4.9, 6.9, 10.0: 1.05, 1.80, 4.9, 6.9, 10.0.

The present full-scale study suggests that GABA receptors in DBA/2 and C57BL/6 mice may contribute to seizure susceptibility. In the present study, we have investigated the relationship between seizure susceptibility and GABA receptors in DBA/2 and C57BL/6 mice. Two enzymes have yielded clear RFLPs. Southern blots of genomic DNA extracted from the livers and cut with a variety of restriction endonucleases were hybridized at least 30 min. Control ejections of vehicle alone, BZ photo-labelled BZBP, or heat-inactivated BZBP did not produce changes in spike frequency observed in response to a 10 s stimulus (n=6 cells). This response to depolarizing current stimuli. A single ejection of BZBP (15-20 lbs/in², 10 s) produced a mean 66% increase in spike frequency. This effect peaked within 5 min of ejection and remained constant for at least 30 min. Control ejections of vehicle alone, BZ photolabelled BZBP, or heat-inactivated BZBP did not produce changes in spike frequency. These results suggest that the 65 kDa BZBP may modulate SFA and that BZ binding to this protein may play a role in the regulation of SFA.

PERIPHERAL-TYPE BENZODIAZEPINE LIGANDS REGULATE SPECIFIC \( \beta \)-PHENTYTOIN BINDING TO RAT BRAIN WHOLE CORTEX MITOCHONDRIA. J. FRANCIS and L. SPERO, Dept. of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 3A8.

These studies were conducted to assess specific binding sites for \( \beta \)-phenytoin (\( \beta \)-DPM) in rat brain and the regulation of these sites in vitro. These sites, previously reported to be concentrated in the fraction of rat brain homogenates, were localized in the mitochondrial fraction but not in synaptosomes. Peripheral-type benzo diazepine ligands (BZs) exhibit high affinity binding to mitochondrial outer membrane, modulated specific \( \beta \)-DPM binding to mitochondrial fractions. P-BZD agonists enhanced both total and \( \beta \)-DPM binding in a dose-dependent manner. The order of BZs was flumazenil \( \alpha \)-BZB: 1.05-1.1, PHT: 1.10, 1.87, 4.9, 6.9, 10.6: 1.05, 1.80, 4.9, 6.9, 10.0. HindIII-cut DNA, when probed with a cDNA representing the C-terminal portion of the alpha subunit, gave the following pattern of bands (approx. sizes in kbp): DZP: 2: 1.10, 1.87, 4.9, 6.9, 10.0: 1.05, 1.80, 4.9, 6.9, 10.0. HindIII-cut DNA, when probed with a cDNA representing the C-terminal portion of the alpha subunit, gave the following pattern of bands (approx. sizes in kbp): DZP: 2: 1.10, 1.87, 4.9, 6.9, 10.0: 1.05, 1.80, 4.9, 6.9, 10.0.

The present full-scale study suggests that GABA receptors in DBA/2 and C57BL/6 mice may contribute to seizure susceptibility. In the present study, we have investigated the relationship between seizure susceptibility and GABA receptors in DBA/2 and C57BL/6 mice. Two enzymes have yielded clear RFLPs. Southern blots of genomic DNA extracted from the livers and cut with a variety of restriction endonucleases were hybridized at least 30 min. Control ejections of vehicle alone, BZ photo-labelled BZBP, or heat-inactivated BZBP did not produce changes in spike frequency observed in response to a 10 s stimulus (n=6 cells). This response to depolarizing current stimuli. A single ejection of BZBP (15-20 lbs/in², 10 s) produced a mean 66% increase in spike frequency. This effect peaked within 5 min of ejection and remained constant for at least 30 min. Control ejections of vehicle alone, BZ photolabelled BZBP, or heat-inactivated BZBP did not produce changes in spike frequency. These results suggest that the 65 kDa BZBP may modulate SFA and that BZ binding to this protein may play a role in the regulation of SFA.
412.9 REGULATION OF THE GABA RECEPTOR COMPLEX IN BRAIN FOLLOWING LIMBIC SEIZURES BY KAINEIC ACID, Patricia Edgar*, Mark A. Rowe and Rochelle D. Schwartz. (SPON: T.A. Slotkin), Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Systemic kainic acid (KA) was used to induce limbic epilepsy in rats. The effects of seizure activity on regulation of the GABA receptor-gated Cl⁻ channel were determined by measuring GABA receptor-gated Cl⁻ flux in rat brain vesicles and binding of (35)S-flurane to "convulsant" sites associated with the GABA receptor/ionophore. Rats were injected with KA (5-20 mg/kg, ip) and sacrificed 2 hrs later. Within this time period initial decreases in locomotor activity followed by catalepsy, wet dog shakes, and finally limbic motor seizures were observed. KA-induced seizure activity decreased maximal muscimol-activatable 36Cl⁻ uptake in cerebral cortical synaptoneurosomes by 12% (p<0.0053, n=9). The distribution of "convulsant" sites labeled by flurane was measured using in vitro receptor autoradiography. KA-induced seizure activity caused a significant decrease in flurane binding in frontal-parietal cortex (15%, p<0.02), inferior colliculus (52%, p<0.001), and molecular and granular layers of cerebellum (43%, p<0.001, and 52%, p<0.001, resp.) These data suggest changes in CA3-induced limbic motor seizures can decrease the activity of the GABA receptor-gated ion channel and convulsant binding in the specific brain regions. Supported by NIH grant NS 24577 and PMF Award to RDS.


The interruption of intracortical, chronic GABA infusion gives rise to an epileptogenic process, which has been named the "GABA withdrawal syndrome (GWS)" (Brain Res 442:175,1988). After induction of a GMS (EEG spikes and myoclonus) in vivo, we prepared slices and recorded neuronal activity located in the vicinity of the infused site. Membrane potentials of more than 60mV and Na⁺ action potentials of 70-110mV indicated that neurons studied were not injured by the experimental procedure. Electrical stimulation of the underlying white matter induced 15-30mV, 80-150msec paroxysmal depolarization shifts in virtually all neurons, indicative of epileptiform activity. Both applications of GABA (0.3-10 µM) and lidocaine (300 µM) depolarized (non-synaptic) depolarization shifts and bursts of action potentials were induced by depolarizing current injections. These results indicate a correlation between cortical GABAergic dysfunction and epileptiform activities at the cellular level following increased and prolonged GABA exposure in vivo.

412.11 EFFECT OF LIDOCAINE ON INHIBITION IN THE RAT HIPPOCAMPAL SLICE. R. Engin and R. Capek. Dept. of Pharmacol. and Therapeutics, McGill University, Montreal, Quebec, H3G 1V6, Canada.

Depression of inhibitory pathways is assumed to be responsible for the seizures induced by local anesthetics. We studied the effects of lidocaine on inhibition in the hippocampal slice using paired-pulse tests. The population spikes (PSs), evoked by orthodromic stimulation and recorded in the CA1 region, were inhibited by conditioning stimuli, either orthodromic (ortho-ortho) or antidromic (anti-ortho). Lidocaine (50 to 300 µM) administered by perfusion produced a concentration-dependent depression of the unconditioned PS. In the anti-ortho test, 100 µM lidocaine showed no significant depression. In the ortho-ortho test, reduced inhibition was also seen at 100 µM lidocaine. 200 µM lidocaine was occasionally enhanced and prolonged the inhibition. No multiple PSs were observed during lidocaine perfusion. Although this data do not contradict the suggestion that the hippocampus is probably not the site of origin of seizures induced by local anaesthetics (Shurr et al., Anesthesiology, 50, 1986), hippocampal disinhibition can play an important role in generalization of seizures induced by lidocaine in vivo.

(Blood by the MRC of Canada)

413.1 COMPUTER ANALYSIS OF TWO METHODS OF MEASURING BLOOD BRAIN BARRIER TRANSPORT KINETICS. Richard A. Hingson and George A. Oyster. Dept. Anesthesiology, Milton S. Hershey Medical Center, Hershey, PA 17033.

A computer model of blood-brain barrier transport was used to compare the ability of two experimental techniques, the brain uptake index technique and the in situ brain perfusion test, to accurately measure blood-brain barrier transport kinetics. The model allows estimates of the Kₘ and Vₐₘₚ can result from both methods. The apparent kinetic constants are critically dependent on a variety of factors including the range of concentrations used, the time of the experiment, the endogenous concentration of substrates and the Vₐₘₚ-to-Kₖᵢ ratio. The results of neutral amino acid transport experiments are particularly sensitive experimental time and the concentration range examined. Supported by NS 16737.

413.2 TIME COURSE OF THE PENETRATION OF SYSTEMICALLY ADMINISTERED IgG INTO THE RAT NEONATAL BRAIN PARENCHYMA. Roderick H. Fabian* & Claire E. Rudek*.*Department of Neurology and +Anatomy and Neurosciences and the +Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas, 77550.

Insight concerning CNS development has been gained by examining the effect of anti-neuronal antibodies on development. We undertook this study to determine whether or not systemically administered xenogeneic IgG crosses readily into the neonatal rat brain parenchyma. Sixteen to 2 day old rat pups were compared to 12 mature rats (150-300g) studied previously. Rats were injected intraperitoneally with purified rabbit IgG (1.3 g/kg) or were left uninjected as controls. After 2, 6, 12, 24, and 48 hours, they were deeply anesthetized and perfused, and the neuraxis was removed and examined for the presence of rabbit IgG using a controlled immunohistochemical technique. Rabbit IgG titers were determined using an ELISA technique. Sections from pups injected with rabbit IgG stained diffusely and strongly positive for rabbit IgG after 6 hours, unlike sections from mature rats which showed no staining except for specific cell groups and the circumventricular organs. Controls showed no staining. Rabbit IgG titers in rat pup serum were maximal at 2 hours after injection. This study supports the assertion that systemically administered xenogeneic IgG reaches significantly higher concentrations in the brain parenchyma of the neonatal rat than in the mature rat. Supported by NIH grant NS 11255.
MONOCLONAL ANTIBODY CROSING BLOOD-BRAIN BARRIER BINDS BRAIN ACETYLCHOLINESTERASE. S. Brimijoin, M. Baumann*, P. Hammond, and V. A. Lennon. Deps. of Pharmacology, Immunology and Neurology, Mayo Clinic, Rochester MN 55902.

As a model for a monoclonal antibody (mAb) antibody, IgG was added to perfusate containing free Pb-203. Pb-203 INTO BRAIN AND CEREBROSPINAL FLUID (CSF). V.A. Hammond*, and V. A. Lennon. Depts. of Pharmacology, Immunology and Neurology, Mayo Clinic, Rochester MN 55902.

Metals such as Pb and Cd have been shown in numerous investigations to be neurotoxic. However, little is known of how these metals cross the blood-brain barrier (BBB). Muscarinic receptors exist on cerebral blood capillaries. (Supported by USPHS Grant NS11050 and NS-13742.)

THURSDAY AM

BLOOD/BRAIN/NERVE BARRIER II

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Metals such as Pb and Cd have been shown in numerous investigations to be neurotoxic. However, little is known of how these metals cross the blood-brain barrier (BBB). Muscarinic receptors exist on cerebral blood capillaries. (Supported by USPHS Grant NS11050 and NS-13742.)


All kinetic studies were conducted with the lateral or fourth ventricular choroid plexus (LVP; FVCP) of the rabbit. At L-carnitine concentration of 10 µM, the uptake was increased linearly for the first 10 min.


Total brain water is regulated during acute hypernatremia based on the separate regulatory responses of extracellular water (ECW) and intracellular water (ICW) to this osmotic stress in the cerebral cortex of anesthetized Sprague-Dawley rats. Plasma [Na+] was elevated by a single ip injection of NaCl (1.25 M, 2 ml/100 g) suggesting that water and electrolytes were determined, in a separate series of experiments, as functions of plasma [Na+]. ICW could then be estimated as the difference between ICW and ECW (mean ± SEM) = 34.5 ± 0.9% and 4.0 ± 3.3% in control cortex were 0.86±0.02 and 2.93±0.02, respectively. ICW decreased by an average of 60% after 2 hours of hypernatremia, whereas ICW remained stable. Plasma [Na+] decreased from 141 to 172 meq/liter, tissue Na content by 25%, and Cl content by 42%. K content decrease in ICW, together with simultaneous increases in tissue Na and Cl content, indicate that a shift of extracellular fluid into brain cells contributes to regulation of ICW during acute hypernatremia. Supported by NIH grants NS-11050 and NS-13742.
413.9

USE OF BIOMATERIAL AND FOUSSIEMIDE TO TEST FOR Na,K,Cl CO-
TRANSPORT IN THE ISOLATED RAT CHOROID Plexus. C. Johnson,
D. Bairamian, J. Parmenter, S. Sweeney and M. Epstein. Depar-
tment of Clin. Neurosci., Prog. in Neurosurg., Brown Univer. and
R.I. Hosp., Providence, RI 02903. Supported by NIH grant NS21062

The regulation of pH, volume and secretion in the choroid
plexus-CSF system is becoming clearer as the nature of Cl
transport in the plexus (CP) is elucidated. Cl-HCO3 exchange in the CP is established; less clear is the pos-
itive functional role of Na,K,Cl co-transport. We tested for
co-transport by analyzing the kinetics of Cl-36 and Na-86 (potassium) uptake by lateral ventricle CP incubated in artif-
cial CSF (37°C). Choroidal tissues were excised from a-
dult Sprague-Dawley rats, homogenized (Morton anaerobia) and preincu-
bated for 20 min in CSF (with drug but without tracer) be-
fore transfer to tracer-containing CSF. The rapidity of Cl
uptake, i.e., Na,HCO3 uptake for steady state, suggest carrier-
mediated uptake "uphill" into the choroidal epithelium. Bu-
matone and fuossemide, inhibitors of Na,K,Cl co-transport, reduced by 25-35% the transport of Cl-HCO3 into the plexus incubated in CSF with drugs (10-6 to 10-8 M). Over the
same concentration range, these loop diuretics decreased tracer Ra (K) accumulation by up to 25%; such findings were corroborated by choroidal tissue analysis of single K (by flame photometry). The ability of bumetanide to sup-
press both Cl transit and K transport in the CP is evidence for co-
transport and suggests its usefulness as a probe in CNS
cerebrovascular permeability. (blood-brain barrier) and CSF transport models.

413.10

THE EFFECT OF LARGE NEUTRAL AMINO ACIDS ON THE
PHARMACOKINETICS OF L-LEVOADO (PD) IN CSF. J.B. Liber,
Depts. of Neurology and Biochemistry, Oregon Health Sciences
Univ. and The Oregon Regional Primate Research Center,
Portland, OR 97201.

The transport of levodopa from blood to brain occurs via
the large neutral amino acid (LNAA) transport system. Because the Kn of LNAA transport approximates the plasma
concentration of LNAA's, competitive inhibition of levodopa influx into brain may result from physiologic
increases in plasma levels after ingestion of protein. This may account for the effects of meals on the response to
levodopa in patients with Parkinson's disease (PD) who exhibit the fluctuating motor response "on-off". In PD patients with 'on-off' an oral load of a LNAA blocks the clinical response to a therapeutic level of levodopa maintained by a constant I.V. infusion. Using a similar
approach in rhesus monkeys, we examined the effect of a
leucine load given I . F. on the disposition of levodopa and
other amino acids in cisternal CSF as a more direct
indication of the effect of LNAA's on the influx of
levodopa into brain. Leucine loading produced changes in
CSF levels of LNAA and levodopa as well as other amino
acids. An unexpected result was a 4-6 fold increase in CSF
glutamate levels.

Supported by NIH grant NS21062 and the Medical Research
Foundation of Oregon.

413.11

PARAVASCULAR FLUID MOVEMENT AFTER MICROEMBOLIC DISRUPTION
OF THE BLOOD-BRAIN BARRIER. D.B. Remmers. Departments of Neurology and Anatomy, Univ. of
Md. Sch. of Med., Balt., MD 21201.

Embolization of the rat brain with polystyrene micro-
spheres (MS) results in focal opening of the blood-brain
barrier (BBB). At these sites, leakage and spread occurs
of macromolecules into brain parenchyma. These findings
provide a basis for understanding brain inflammatory changes in brain
in chronic hypertension. (Supported in part by PHS NS 16332)

413.12

A RAPID, SENSITIVE METHOD FOR QUANTITATION OF
ESTRADIOL CONJUGATES IN BLOOD TISSUE AND CSF ADMINISTRATION OF A BRAIN-ENHANCED DELIVERY SYSTEM FOR ESTRADIOL. M.

Brain-enhanced delivery of Estradiol (E2) with an E2
chemical delivery system (i.e. estradiol 17β-(1-hy-
dihydroxytriglycollinate)) creates a problem for separating and quantitating brain levels of the active
forms of E2 and its conjugates. The first step uses a water:acetone
(50:50, v/v) solvent system to extract the conjugate from biological
tissues (e.g. rat brain); second, the conjugates are hydrolyzed in IN NaOH; third, solid phase extraction (SPE) with C18 reverse phase column is used to isolate E2 and the final step is the RIA of E2.

The application of this procedure to biological tissues was
assessed using rat brain homogenates. Recovery of the E2,
after solvent extraction and SPE chromatography, is 101 ±
4.5% while recovery of the E2 conjugate is 64 ± 2% with
greater than 80% hydrolysis of the conjugate. Since the
solvent extraction, hydrolysis, and SPE steps can be
completed for 100 samples in one day, this procedure
represents a rapid, reliable and practicable method for
the quantitation of E2 and its conjugates in tissue
samples (Supported by NIH HD 22540).

413.13

CEREBROVASCULAR PERMEABILITY IN EXPERIMENTAL HYPERTENSION.
P.A. Grady. Univ of Maryland Sch of Med., Balt, MD 21201.

Cerebrovascular permeability remains unaltered in most
physiological states due to intact blood brain barrier. This
barrier has been shown to be breached under condi-
tions of acute hypertension but chronic hypertension is less clear. This study tests the effect of chronic hypertension on cerebrovascular permeability.

Goldblatt's rat was performed for 3 months. Blood pressures ranged from 135-165 mm Hg systolic arteri-
olar pressure. After 3 months, these rats and controls were anesthetized with chloral hydrate 100 mg/kg IP. Horse-
red peroxidase (HRP) Sigma Type II or VI was injected iv and allowed to circulate for periods of 5, 10, or 20
minutes. Animal were perfused with fixative. Brain sections were incubated with diaminobenzidine and tetramethylbenzi-
dine and examined at the light microscopic level. Patchy areas of HRP were scattered diffusely across grey and white matter areas. TMB incubated sections showed increased staining over the rostral portion of the basal ganglia, basal forebrain and pontine areas of brainstem predominantly.

The results of this study suggest that there is an increased cerebrovascular permeability under conditions of chronic hypertension which allows for the passage of macromolecules into brain parenchyma. These findings provide a basis for understanding brain inflammatory changes in brain
chronic hypertension. (Supported in part by PHS NS 16332)

413.14

DUAL ROLE OF ENDOTHELIAL CELL TUBULES IN BLOOD-BRAIN
for Bas. Res. in Dev. D iseases, Poughkeepsie, NY 12601.

The present studies extend our exploration of the ultrastructural features of tubular profiles associated in
endothelial cells (ECs) following blood-brain barrier (BBB) insult. We have reassessed several of our previous
experimental paradigms in attempts to elucidate the functional role of these tubular profiles. Previous
studies have indicated that the tubules represent either: (1) lymphoendothelial structures derived from the network
of the cell, or (2) serve as purported conduits for transepithelial transport. In several experimental
models in which the BBB was compromised, employing
conventional transmission or high voltage electron micros-
copy, we observed endothelial peroxidase-positive, conduc-
tive-like structures, directly connecting luminal and ablum-
inous cellular surfaces in the thinnest portions of the EC. In wider
regions, tubules were observed in the proximity of, or
bound to monolayers of fibroblasts and pericytes. Inflammatory cells (ICs) by scanning microsoopy were often
observed attached to parajunctional EC regions. Our re-
results suggest a dual functional role of these tubules for
transport and also implicate a possible mechanism for IC attachment and eventual migra-
onto the injured BBB. Supported by NYS ONR and
NIH Grant # RR1219.

Clinical trials utilizing intracerebral (IC) injection of rIL-2 and lymphokine-activated killer (LAK) cells to treat brain tumor have reported temporary exacerbation of neurological deficits and lethargy in most patients. The current study examined the histopathological effects of a single 5µl injection of rIL-2 (12,000 U, CETUS), its excipient or saline into the right parietal lobe of rats. Animals were assessed at various times by transcardiac aldehyde perfusion (1h following IV injection of horseradish peroxidase (HRP)) and brains were processed in the electron microscope. Histological findings at 4h, 12h and 24h post-injection showed traumatic BBB disruption with no differences between injections of rIL-2, excipient or saline. HRP extravasation persisted at 3 and 8 days only in animals injected with rIL-2. In addition, only rats receiving rIL-2 demonstrated increased injection site with leukocytic infiltration, perivascular cuffing and localized edema which was evident at 24h and continued to increase over an 8 day period. Our results suggest that an IC injection of rIL-2 alters the integrity of the BBB directly and/or potentiates and sustains cellular events responsible for barrier disruption following traumatic injury to the brain.


After spinal cord injury, abnormal barrier permeability to the tracer horseradish peroxidase (HRP) and the distribution of cationized ferritin (CF), a marker of anionic sites, were examined in the spinal cord at the ultrastructural level. In control (laminectomized) animals, vessels remained impermeable to HRP. Although the luminal surface of microvessels was evenly labeled with CF, vesicle formation along the luminal front was associated with partial thinning and/or loss of anionic sites. In initial stages of vesicle formation (prior to stalk formation) anionic sites were confined along the longitudinal membrane. With formation of a stalk, negative sites were present along the neck of the stalk with few CF molecules in the CF was absent in vesicles which no longer maintained continuity with the plasma front or which contacted the abluminal front. After injury the distribution of anionic sites, relative to luminal vesicle formation, was similar to that observed in laminectomized animals. However, there was reduced CF binding along the luminal pipelines. This loss of surface charge properties was typically associated with edematous tissue as indicated by enlarged perivascular spaces, swollen astrocytic foot processes and abnormal vascular permeability to HRP. (Supported by NIH NINDS HD1923324 to L.J. Noble).

413.16 CYCLO-FOSPHAMIDE SHORTENS THE LATENCY FOR RADIATION INDUCED BBB BREAKDOWN M.P. Remler and V. Marcussen (SPO: A. Gabor) Dept. of Neurology, VANC, Univ. of California, Davis, Martinez, CA 94553.

Irizing radiation is known to lower the blood brain barrier (BBB) after a dose dependent latency. Just prior to irradiation, the rats were treated with the radiation sensitizer Chlorpromazine 3.75 mg/kg, Cisplatin 5 mg/kg, Cyclophosphamide 100 mg/kg, Acyclovir 100 mg/kg, Bleomycin 3 mg/kg, Doxorubicin 10 mg/kg, and Actinomycin O 0.5 mg/kg and Doxorubicin 0.12 mg/kg. Rats were irradiated with 20 Gy of alpha particles in a single dose focused to a volume of 0.5 cc in the center of the left hemisphere. The BBB was detected in the irradiated portion of the brain by the presence of staining following Evans Blue dye injection. All except cyclophosphamide showed no reduction of the latency of 240 days expected for BBB breakdown following 20 Gy. Of four rats test with cyclophosphamide, one showed no BBB breakdown at 106 days while three showed breakdown at 134, 148 and 169 days. The left hemispheres with lowered BBBs were histologically normal as were the unirradiated control right hemispheres. This research was supported by the Veterans Administration and the USPHS NIH Grant No. NS 17777.

413.18 HOST BLOOD-BRAIN BARRIER PERMEABILITY IS UNALTERED BY INTRAPARENCHYMAL FETAL NEURAL GRANTS M.D. Gaunt, D.O. Mean* (Epsom: JW Bigbee), Med Coll of VA, Richmond, VA 23298.

The permeability of the blood-brain barrier (BBB) may be important in the immunologic response of the host CNS to grafted tissue. There is evidence (Rosenthal, Science 258:772-774, 1987) that vasculature established in block grafts of fetal neural tissue can maintain certain characteristics of normal BBB despite the fact that the barrier is mature (in terms of its permeability to certain molecules) even in the embryo. However, fetal neural cell suspensions injected into host brain parenchyma may not cause this disruption of the BBB, as vascular shunts may originate from the neural graft. Anesthetized adult male Sprague-Dawley (SD) rats received grafts of E15 SD foetal brain. The donor tissue was dispersed into a cell suspension using trypsin (Schmitt et al., Brain Res 279:195-198), then injected electropically into the rostral corpus callosum. The opposite side received a vehicle injection. Survival periods of 14 days (n = 3), 30 days (n = 5), 90 days (n = 4), and 180 days (n = 4) were studied. One hour before the animals were sacrificed, 5 mg/kg of HRP type VI (Sigma) was injected i.v. after 1 mg/kg. Benadryl (diphenhydramine) was given i.v. to prevent anaphylaxis. The anesthetized animals were transcardially perfused with 1% paraformaldehyde, 1.25% glutaraldehyde, and 50 micromolar sodium potassium taken on a vibratome. Thionin stain acetylcholinesterase (ACHE) histochemistry and HRP histochemistry (Mesulam method) were performed.}

413.20 NERVE SEGMENTS FORMED IN SILICONE TUBES RECONSTITUTE A PERINEURAL BUT NOT A VASCULAR ENDONEURIAL PERMEABILITY BARRIER. N.A. Azran, A. Zwikowski, and L.S. Williams (Epsom: JW Bigbee). Dept. of Neuronal Growth and Regeneration, NINICDS, NIH, Bethesda, MD 20892 and CNS Diseases Research (L.R.W.), The Upjohn Co., Kalamazoo, MI 49001.

Peripheral nerve fibers of adult mammals can regenerate through silicone tubes wherein they reform a new nerve segment. In the present study we examined these segments to determine whether endothelial and perineurial sheath cell proliferation and matrix also reconstituted. Normal barriers, these barriers, with tight intercellular junctions, regulate the movement of macromolecules into the endoneurium from inside and around a nerve. Adult rats had their sciatic nerve cut bilaterally, and the proximal and distal ends sutured into 10 mm long tubes that were filled with saline. One tube was removed after one month, a time when a cable of tissue extended through the entire tube. The permeability of the blood and perineurial barriers was examined separately, with the tracer horseradish peroxidase (HRP; MW 40,000). The reformed nerve segment was excised or free of the tubes, contained remodeled axons, blood vessels and perineurial cells. However, in contrast to normal or distal regenerated nerve segments, were mini-compartmentalized by perineurial-like cells. All the regenerated blood vessels were excluded from the endoneurium of these nerve segments. Additionally, all the regenerated vessels became impermeable require investigation.
ROLE OF CALCIUM DEPENDENT INFLUX IN NEURONAL DIFFERENTIATION. DEVELOPMENTAL PERIODS AND EVOLUTIONARY STATE. INTERNAL CALCIUM IS CORRELATED WITH A PERIOD OF SENSITIVITY TO EXTERNAL CALCIUM. Janet Holliday and N.C. Spitzer. Dept. of Biology, University of California, San Diego, La Jolla, CA.

Calcium dependent action potentials (Atp) can be evoked from Xenopus spinal neurons cultured from neural plate stage embryos from the time they can first be recorded, but neuronal maturation during the first day of development is periodic in dependence of this ATP shifts from calcium to sodium (Spitzer and Lamborghini, 1976). Growth of cells under conditions which prevent calcium entry produces alterations in normal patterns of differentiation (Ryan and Spitzer, 1984; Henderson, Smith and Spitzer, 1984). These studies suggest that calcium influx may have a significant role in development.

The role of spontaneous calcium influx was investigated by monitoring changes in the levels of intracellular calcium during the first day in culture using the fluorescent calcium indicator, fura-2. Spontaneous elevations in internal calcium concentration are evident in the largest percentage of cells during the 6th to 12th hours in culture. This is the period during which calcium dependent Atp can be evoked. This is also the period which exhibits the highest sensitivity to the removal of external calcium. When normal culture medium is exchanged for calcium free medium during this period, both neurite length and neurite-motoneuron contacts are altered in the same manner as in cultures which have developed in the absence of calcium for the entire growth period of 24 hours. Spontaneous elevations of calcium are not observed if the cells are acutely exposed to caffeine, an agent known to block calcium channel blockers or in the absence of external calcium. Thus the elevation of internal calcium is likely to be due to calcium influx. Many of the cells which score as spontaneously active proceed to develop into well differentiated neurons.

This study was supported by NIH grants NS 07383 to DPM and NS 24444 to KGB.

414.3 ORTOGENESIS OF CALCIUM CHANNELS IN A RECENT BRAIN UTILIZING THE ZEBRAFISH, DANIO RERIO, L. Bull Mental Retardation Research Center, University of California, 760 Westwood Plaza, Los Angeles, CA 90024.

One surprising aspect of voltage-dependent calcium channel is by extracting mRNA from brains of different postnatal ages and injecting mRNA into oocytes of Xenopus laevis. Several investigators have investigated the presence of mRNA from rat brains to expression of additional Ca2+ channels in the oocyte, which activate calcium-dependent Cl- channels (Leonard, P. et al., 1987; Schach & Gadsden, 1987). Under these conditions, a current for the Ca2+ channel was recorded from the nucleus of the oocyte from an embryo at 2 days of age to the presence and absence of calcium. After incubation of Ca2+ and Ca2+ currents, oocytes containing brain mRNA of 15-24 day old mice have current levels similar to rat adult males of 75-80 mV (Buchbach and Gadsden, 1987). Similarly, measured current of oocytes injected either with 1-3 day old mouse brain mRNA or water (controls) have much lower values. By applying serotonin to the oocyte bath, a transient inward current was seen only in mRNA-injected oocytes regardless of mouse age. This current has been shown (Dascal, et al., 1986), to involve CI- channels (probably by releasing internal Ca2+ stores), suggesting that CI- channels are present at very early ages. These observations agree with recent studies on development of Ca2+ channels using different assays (cf. Kazazoglou, et al., 1983).


Previous research has demonstrated patterns of transiently expressed acetylcholinesterase (AChE) histochemical staining in the dorsal thalamus and cortical development of rat pups. This transient AChE activity appears to be found within thalamocortical neurons of the ventral basal, dorsal lateral geniculate, and ventral medialis geniculate nuclei. The temporal domain of transient AChE expression corresponds well to the time of development of thalamocortical connections suggesting that transient AChE may play a role in thalamocortical development (Robinson, Neurosci. Lett. 75:29, 1987). The present studies were undertaken to determine whether transient expression of AChE in the thalamus is a common characteristic of developing rodents. Experiments were performed on rats, mice, gerbils, hamsters and guinea pigs. Adult animals and infants 0-25 postnatal days of age were used. Animals were perfused with aldehydes and frozen sections were processed for AChE histochereism. Developing animal of all species showed intense and transient AChE histochemically activity of the ventral basal complex. The adult ventral basal complex of all species displayed minimal AChE activity. Infants of all species showed greater AChE activity in the ventral medial geniculate nucleus than did adults, although only the infant rat displayed any intense ACHE activity. The temporal domain of AChE expression in the ventral medial geniculate nucleus of all species was observed in all species with the exception of mice. In the mouse, AChE expression was found in all species with the exception of mice. In the mouse, AChE expression was found in all species except for mice.

This study was supported by NSF grant 87-08510 and NIH grant NS 28574.


We have previously shown that both insulin and insulin like growth factors (IGF) (Recio-Pinto et al. J. Neurosci. 6:1211, 1986) share with nerve growth factor (NGF) the capacity to induce neuronal survival and stimulate neurite growth in cultured sympathetic and sensory cells. Here we investigated whether these neurotrophic responses could be elicited in spinal cord cultures as well. In vitro, by day 1 of basal media containing 1:11 in 1:5 containing 1:4 4Km contain. About 25-30% of the plated neurons were responsive to insulin. The neurotrophic response has been observed in other cultures of these cells and similar results were obtained with insulin and IGF. The number of neurons with neurites and the average length of neurites were increased. High doses of insulin (10-6m) combined with IGF (40-6m) did not significantly increase neurite outgrowth at 3 days over insulin alone. IGF1 (1m) also enhanced neurite growth. The fact that IGF1 and IGF2 may be important physiological regulators of neurite formation and play a role in the development of the spinal cord. (Supported in part by NIH grant 881 NS24327).

414.6 GAP JUNCTIONS DURING NEOCORTICAL DEVELOPMENT: NORTHERN BLOTTING AND IMMUNOCYTOCHEMICAL STUDIES. C.C.G. Naus, D. Feinlein and G.M. Kidder*. Departments of Anatomy and Zoology, University of Western Ontario, London, Canada; Research Institute of Scriver Clinics, La Jolla, CA.

The developmental appearance of gap junctions was examined during postnatal development of the rat neocortex. A cDNA specific for the gap junction protein GJ protein (connexin) was used to probe Northern blots of total RNA isolated from the neocortex of rats at postnatal day 4, 7, 11, 15, 19 and 25. From postnatal day 4 to 15, very high levels of gap junction mRNA are detected. In the adult rat, gap junction expression does not occur in the level of this mRNA. In order to examine the distribution of gap junctions at the cellular level, sections of neocortex at these developmental stages were immunocytochemically stained with a polyclonal antibody to connexin. Immunoreactivity for gap junctions is present in association with neurite outgrowth at about 2 days after E5-15/20% of neurons. The number of neurons with neurites and the average length of neurites were increased. High doses of insulin (1m) combined with IGF (10-6m) did not significantly increase neurite outgrowth at 3 days over insulin alone. IGF1 (1m) also enhanced neurite growth. The fact that IGF1 and IGF2 may be important physiological regulators of neurite formation and play a role in the development of the spinal cord. (Supported in part by NIH grant 881 NS24327).


1041.9 IMMUNOCYTOCHEMICAL IDENTIFICATION OF INTERMEDIATE FILAMENT PROTEINS IN THE DEVELOPING NERVOUS SYSTEM. R. C. Sze2ro and H. Gainer. Lab. Neurochem., NINCDS, NIH, Bethesda, MD 20892.


1041.11 AUTORADIOGRAPHIC ANALYSIS OF CHICK RETINAL DEVELOPMENT IN CULTURE VERSUS IN Utero. S. G. Spencer* and J. A. Robson. Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.


The retina of the larval lamprey consists of a mature central retina and a relatively undifferentiated peripheral retina. The histological and functional differentiation of the peripheral zone is completed during metamorphosis (Rubinson, et al., '77; Rubinson and Cain, '83). In retinal differentiation to the size of the animal and, thereby to its age, indicates that differentiation of the lamprey retina spans at least five years and may not be a continuous process.

Specimens with a histologically undifferentiated peripheral retina ranged in length up to 77 mm (n=9, mean=64.2). Specimens in which neurons and ganglion cells were clearly distinguishable peripheral ranged from 68 to 90mm in length (n=4, mean=77.3). The differentiation of other cells was seen in lamprey of this size. In the adult lamprey, the photoreceptor inner and outer segments were not only in animals entering metamorphosis.

Thus, ganglion cells may appear when the lamprey is 2 years old and differentiation of the remaining neuroblasts requires a minimum of 2 additional years. Beyond 4 years of age, the mean length of the ganglion cell does not change. As the age at metamorphosis ranges from 5 to as much as 13 years, it seems that the completion of the retina can be deferred for a number of years.

Changes in cellular composition and in volume of the vescalocular organs (VNO) of embryonic and fetal garter snakes were examined. Microprojections drawings of 12 microns serial sections were used to calculate the volumes of the supporting cell (SC) and bipolar cell (BP) layers in VNOs. The SC layer decreased to a quarter of its volume from mid-gestation to the period just before birth. During the same period of time, the BP layer decreased by 2.5 times its volume. At birth, the volume of the SC layer has not changed, but the volume of the BP layer has increased by 2.5 times. Experiments using thymidine autoradiography suggest a differential generation of cells in the SC and BP layers of the VNO. Supported by NIH grant NS 11713.

414.14 DOES THE MOUSE BRAIN HAVE ITS OWN MACROPHAGES? Hoo, C. * Richardson, A. * and Pedcroft, S. Department of Anatomy, University of Saskatchewan, Saskatoon, SK, Canada.

When astroglia culture were exposed to unfavorable growth conditions, large numbers of macrophages appear. These cells are ameboid, highly phagocytic, and excrete lysozyme. In embryonic stages, cultures initiated with mouse embryos at Thaller stages T14(E9), T15(E11), T14(E13), T15(E15), T23(E17) and from newborn animals, when grown for an additional 10 days without medium change, macrophages were present and the medium contained lysozyme. In contrast, cultures that were fed no, or only a few macrophages, and the medium, contained no, or very low concentrations of lysozyme.

Macrophages in neonatal astroglia cultures were compared with macrophages from adult mouse spleen and peritoneal cavity on the basis of their growth characteristics, response to trophic factors, presence of α-naphthyl butyrate esterase, peroxidase, Mac-1, Mac-3, ML/70, Ia, Dil-ac-CLD, GFAP and vimentin. We conclude that macrophages in the neonatal astroglia cultures share many macrophage-specific characteristics with spleen and peritoneal cavity macrophages, but that some notable differences exist, indicating the presence of a specific type of macrophage in mouse brain. Precursors of these macrophages are present in the astroglia cultures in brain development. This work was supported by MRC of Canada Grant MT4325.
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One hundred and fifty two patients with unilateral temporal- or frontal-lobe excisions and 41 normal control subjects were tested on four kinesthetic recall tasks. The first two studies required subjects to monitor peripheral feedback to determine their distance or the on-set position of examiner-defined arm movements. In the next two tasks, the subjects were asked to retracethe movements to be recalled, and hence reliance on feedback was reduced. Temporal lobectomy did not interfere with performance, except on the examiner-defined kinesthetic location task where left or right large hippocampal resection produced an impairment following 30 s of counting. Patients with left frontal-lobe or small right frontal-lobe excisions performed all tests normally, but those with large right frontal-lobe removals were impaired on the examiner-defined tasks across all delay conditions, and with both hands. The results point out an important role played by the right frontal lobe in the monitoring of kinesthetic feedback both during the presentation of the movements and during the recall attempt.

INDEPENDENCE OF MEMORY FUNCTIONS AND EMOTIONAL
BEHAVIOR: SEPARATE CONTRIBUTIONS OF THE HIPPOCAMPAL
FORMATION AND THE AMYGDALA. P. Alvarez-Ropero, M. Messchas,
Group in Neurosciences and Dept, of Psychiatry, U.C.S.D., La
Jolla, CA 92093, and V. A. Med. Ctr., San Diego, CA 92161.
Monkeys with large medial temporal lobe lesions, including the hippocampus and surrounding cortical areas (H+ lesion), are impaired on tasks sensitive to human amnesia. However, damage to the areas included in the H+ lesion can also cause changes in emotional behavior (e.g. the Kluver-Bucy syndrome). One possibility is that memory might be affected by these changes in emotional behavior. To examine this possibility, we compared the performance of monkeys with extensive or partial lesions of the medial temporal lobe on two behavioral batteries. The first measured performance on four different memory tasks. The second measured emotional behavior by evaluating responses to a variety of inanimate objects (e.g. a model of a snake, a black rubber boot, M&M candy).

Monkeys with H+ lesions were impaired on the memory tasks, and also showed abnormally elevated emotional reactivity. Monkeys with selective and circumscribed amygdala lesions performed the memory tasks normally but showed elevated emotional reactivity. Monkeys with damage to components of the hippocampal formation but without amygdala damage were impaired on the memory tasks but showed normal emotional reactivity. This double dissociation shows that memory impairment is independent of the observed changes in emotional behavior. Implications of these findings for the function of the hippocampal formation and the amygdala will be discussed.

TRANSIENT GLOBAL AMNESIA: CHARACTERIZATION OF
RETROGRADE AMNESIA IN SIX PATIENTS: M. Krivetshevsky* and
L.R. Squire (SPON: N. Butters), V.A. Medical Center,
San Diego, CA 92161, Deps. Neurosciences and
Psychiatry, U.C.S.D., La Jolla, CA.
Transient global amnesia (TGA) is a short-lasting neurological condition in which memory impairment is the predominant deficit. We studied six episodes in 1987, during and after their episode. During TGA, all patients were impaired on tests of new learning ability for verbal and nonverbal material. Additionally, during the episode, patients had difficulty recalling news events from the time period 1960-1985. Recall for events from 1950-59 was similar during and after TGA. During memory for news events was impaired for the time period 1980-1985 but not for more remote time periods. Memory was normal before and after TGA. Results from a personalized test of past memory given during TGA similarly suggested that retrograde amnesia, albeit patchy and varying with some prominent positive memories which had occurred as long ago as 1960. The memory impairment exhibited by these patients will be compared to the memory impairment exhibited by other patients with chronic amnesia.

AMNESIA FOLLOWING MEDIAL TEMPORAL LOBE DAMAGE IN MONKEYS:
THE IMPORTANCE OF THE HIPPOCAMPUS AND ANGIODAMYALID
REGIONS. S. Zola-Morgan, L.R. Squire, and D.C. Amatul.
V.A. Medical Center, San Diego, CA 92161, Dept. of
Psychiatry, U.C.S.D., La Jolla, CA 92093, and the Salk
Institute, San Diego, CA 92138.
Monkeys with bilateral medial temporal lobe lesions that damage the hippocampal formation, amygdala, perirhinal cortex, and parahippocampal cortex (H+A lesion) are more severely impaired on tasks such as delayed matching to sample (DNMS), which are sensitive to human retrograde amnesia, and a more restricted lesion of the hippocampal formation that includes much of the parahippocampal cortex (H lesion) produces a significant retrograde amnesia. In order to determine what damage in the larger H+A lesion accounts for the more severe memory impairment, we prepared three groups of operated animals. Animals with stereotaxic lesions limited to the perirhinal and parahippocampal cortices were damaged bi-laterally without direct involvement of the amygdala or hippocampal formation, animals were at least as severely impaired on DNMS. Moreover, the addition of a selective amygdaloid lesion to the H lesion did not increase the impairment associated with the H+ lesion. However, when both the perirhinal and parahippocampal cortices were damaged bi-laterally without direct involvement of the amygdala or hippocampal formation, animals were at least as severely impaired on DNMS as those with H+A lesions. Since the perirhinal and parahippocampal cortices provide the major input to the moniker hippocampal formation, these data emphasize the importance of the hippocampal system in temporal lobe amnesia.

Information is often recalled in conjunction with its source (i.e. when or where the information was learned). Previous studies showed that some amnesic patients exhibit source amnesia: they can recall a few facts taught in a recent session but they attribute the information to some other context. This study investigated the ability of patients with frontal lobe lesions and two groups of control subjects (elderly and young) to learn new facts as well as to recall the source of the information. In two experiments, patients with frontal lobe lesions recalled facts as well as the control subjects. However, both patients with frontal lobe lesions and elderly control subjects were more likely than young subjects to attribute facts learned within a test session to an incorrect source. In addition, patients with frontal lobe lesions but not the elderly or young control subjects sometimes attributed facts to the test session itself, when these facts had actually been known to the subject prior to the test session. This study shows that source errors can occur in patients with frontal lobe lesions, as well as in healthy elderly subjects, in the absence of amnesia. The frontal lobes may play a special role in helping join information in memory to the context in which it was learned.


We compared the performance of 9 Korsakoff (K) and 9 alcoholic control (AC) subjects on a series of chromatonic tasks using CR task presentation and a manual response board. Tasks included reaction times (rt) facilitated by stimulus driven, subject driven, and voluntarily controlled shifts in attention as well as Sternberg-type short term memory retrieval and sequential finger tapping. Korsakoffs exhibited slower rt's in all tasks. Although the K's showed normal superiority of rt's to valid as opposed to invalid cues, they were significantly slower on subject driven than stimulus driven attentional tasks, a pattern opposite that observed among controls. While the AC's were able to utilize cues in the voluntarily driven attentional task at a delay of 250 msec, K's showed no such benefit until a delay of 1000 msec. On both the memory retrieval and motor sequencing tasks, K's were consistently slower for all response conditions. The speed of retrieval of items from short term memory correlated significantly with performance on the attentional tasks.

418.10 Amnesics' Relatively Preserved Recognition in Implicit Memory. William Hirst,† Elizabeth A. Phelps*, Marcia K. Johnson, and Bruce T. Vole. (SPON: F. McDowell.) Graduate Faculty, New School for Social Research, NY, NY, Princeton University, Cornell University Medical College.

Several theorists have divided the memory system into a component responsible for the explicit retrieval of declarative memories and a component responsible for the implicit retrieval of procedural memories (Squire, 1986). This dichotomy is supported in part by finding that humans anterograde amnesia disrupts explicit declarative memory while leaving implicit procedural memory intact. Recently, it has been reported that amnesic recognition is relatively preserved when compared with amnesic recall. In as much as recognition is an explicit declarative memory task this result could suggest that aspects of amnesic explicit memory remain intact. However, amnesics' unexpectedly good recognition could build solely on their intact implicit memory. Three experiments test this hypothesis. In two, amnesic recognition is raised to normal levels even in the absence of any signs of priming as measured by stem completion and perceptual identification. In the third, factors affecting amnesic recognition such as depth of processing are shown to have no effect on amnesic priming, suggesting that amnesic recognition and priming involve different mechanisms. A model accounting for the results is discussed.


Neuropsychological paradigms have indicated the presence of frontal lobe dysfunction in schizophrenia. However, the matter of task difficulty has not been adequately addressed. In this paper, patients might do worse on these tasks simply because they are harder. Also, prefrontal cognitive failure might reflect "downstream" dysfunction in the basal ganglia. We attempted to examine these issues by using variants of the Tower of Hanoi puzzle. A three disk version is thought to emphasize problem solving and has been found to be sensitive to frontal lobe dysfunction while more difficult four disk version is thought to emphasize procedural learning and is sensitive to basal ganglia disease. Relative to controls, schizophrenic patients performed as poorly on the easier three disk version as they did on the open four disk versions. Moreover, in repeated trials, patients were able to learn the four disk tower at a rate similar to that of normal subjects, though they learned the three disk tower much more slowly. These results suggest that poor performance on frontal lobe tests is not simply a function of task difficulty and that certain aspects of basal ganglia function may be relatively more intact in schizophrenia than frontal lobe function.


We have studied the photoincorporation of the hydrophobic probe [[**]IITID] into the AChR of T. californica boutons associative synaptic membrane. [[**]IITID] labeling of the o-subunit of the AChR. Two regions of the o-subunit incorporate label as determined by digestion with S. aureus V8 protease: the first, defined by a 20 kDa cleavage fragment, begins at residue Ser-1 and contains the third cysteine of the TM1-M3 region, the second extends from Asn-339 through the hydrophobic segment M4. Two fragments, spanning most of the amino terminal region from cell-attached patches, gradually disappeared from outside-out patches over few minutes. Interestingly, the receptors, which were stable in channel conductances of 2OpS. Interestingly, the receptors, which were stable in cell-attached patches, gradually disappeared from outside-out patches over few minutes; this suggests that nucleotideregulatory factors may modulate the function of these neuronal nicotinic receptors.


Recently, a family of genes encoding proteins related to muscle endplate nAChR subunits has been isolated from the avian genome and sequenced (Neuf et al., EMBL J., 7, 1998/1999). These genes (and their protein products) have been termed a 2, a 3, a 4 and a 5 (non-2) and are expressed in the central and peripheral nervous system. All neuronal alpha subunits possess the pair of vicinal cysteines that thought to be part of the ACh binding site, whereas these cysteines are lacking in the non-2. In this study, we used voltage clamp and patch clamp techniques to measure the physiological properties of nicotinic ACh receptors expressed by oocytes 1-3 days after nuclear injection with cDNA for a 3 and a 5 linked to a heat-shock promoter. The DNA injection technique was used because we found that it gave more reliable ACh receptor expression compared to oocytes injection with cDNA. Heat-treated oocytes injected with both a 4 and a 5 constructs expressed up to 2-3mA of ACh-induced currents that could be reversibly blocked by hexamethonium but that were insensitive to bungarotoxin. From dose-response experiments, we determined that the slope of log-response vs log-concentration plots up to 0uM ACh was 1.5, suggesting that the functional receptors assembled with at least two a 4 subunits. Single channel measurements of receptors from outside-out patches demonstrated that these receptors had linear 1-V curves, reversal potentials of about -7mV and single channel conductances of 20PS. Interestingly, the receptors, which were stable in cell-attached patches, gradually disappeared from outside-out patches over few minutes; this suggests that other nucleotideregulatory factors may modulate the function of these neuronal nicotinic receptors.
ION CHANNELS: L IG A N D -G A T E D II

419.3 DIFFERENCES IN THE PHOSPHORYLATION OF UNASSOCIATED, ASSEMBLED BUT CYTOPLASMICALLY AND SURFACE ACYLTYLATED AChR RECEPTORS. W.N. Green and T. Claudio. (SPON: R. Wyman) Dept. of Cell. and Molec. Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510

We have recently shown that Torpedo nicotinic acetylcholine receptor (AChR) subunits were studied using a mouse fibroblast cell line in which the four Torpedo AChR subunit cDNAs had been stably integrated into the host cell genome (Guze et al. Science 238:1688, 1987). Although each of the subunits is synthesized, glycosylated, assembled into AChR complexes, and the complexes are expressed on the cell surface where they display all of their proper pharmacological and physiological properties. Intact AChR-fibroblast cell lines were first incubated with [32P] and unlabeled α-bungarotoxin (BuTx), then solubilized, followed by sequential immunoprecipitations with BuTx-specific or subunit-specific antisera. These different precipitations resulted in the isolation of the four possible AChR subunits: 1) unassembled, cytoplasmic subunits, 2) assembled, cytoplasmic AChR complexes, and 3) assembled, cell surface AChR complexes. The degree and pattern of AChR phosphorylation differed among these three AChR pools. Phosphorylation of the y and β subunits was observed in the unassembled subunits and the surface AChR complexes, whereas only the δ subunit (and possibly β) was phosphorylated in the cytoplasmic AChR complexes. No phosphorylation of the α subunit was detected in any of the AChR pools. The cAMP-dependent protein kinase stimulators forskolin, cAMP-S, and cholera toxin, increased phosphorylation of the unassembled and cell surface AChR pools with no corresponding increase in the phosphorylation of the cytoplasmic assembled AChR pool. Further analysis of the role phosphorylation may play in AChR assembly and/or function is continuing.


Cloning of the muscle nicotinic acetylcholine receptor (AChR) α, γ, and δ subunits cDNAs from Xenopus laevis stage 17 or 22 cDNA libraries has been reported (Baldwin, T. et al., J. Cell Biol. 106:469, 1988). We report here, the isolation of a fourth putative AChR subunit (Xenopus muscle α2, xα2) from a stage 17 embryonic Xenopus cDNA library. We have tentatively assigned xα2 as an α subunit for the following reasons. Expression in a rabbit reticulocyte lysate system of a very short transcript 192 mRNA makes a 40 kd polypeptide that is immunoprecipitated by polyclonal antiserum directed against Torpedo α subunit. The deduced amino acid sequence of clone xα2 revealed that it is composed of 437 amino acids (found in all muscle-like α subunits), it contains conserved cysteine residues compared to α2 and α3 (Torpedo) and α1 (AChR numbering), and it contains the one potential N-linked glycosylation site corresponding to Torpedo residue #141. A comparison of the amino acid similarities (identity plus conservative substitutions) among xα2, Torpedo α, and the Baldwin et al. Xenopus α clone (xα), revealed: ~80% similarity between xα2 and xα, ~75% similarity between xα2 and Torpedo α, ~<50% similarity between xα2 and xα and Torpedo δ; ~<80% similarity between xα2 and xα. The xα2 and xα clones show similarities throughout their entire lengths with the most divergence occurring between the amino terminus and the first putative transmembrane domain, M1. Transcripts from both xα2 and xα are found in adult Xenopus muscle and appear to be coordinately expressed throughout early embryonic development (oocyte, stages 10, 17, 22, 25, 30, 35).

419.6 ACTIVATION AND BLOCK OF SINGLE NICOTINIC RECEPTOR CHANNELS OF THE TORPEDO ENDPLATE BY DECA, D. S. Hartman and T. Claudio. Dept. of Cell. and Molec. Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510

The temperature-sensitive phenomenon is not fibroblast-specific, however it does appear to be Torpedo-specific. Another stable L6 muscle cell line was established in which only the Torpedo α subunit cDNA was integrated (L6-α). Expression of Torpedo-α hybrids in L6-α cells only occurred at temperatures lower than 37°C and in addition, three classes of AChRs could be isolated. AChRs were expressed that contained two rat, two Torpedo, or one rat and one Torpedo α subunit, demonstrating that the α subunit in an AChR complex need not originate from the same polyline.

Further analysis of the Torpedo temperature-sensitive phenomenon suggests that the temperature-sensitive step occurs before assembly and is probably involves an altered polypeptide conformation. 1) Once hybrid or all-Torpedo AChRs are formed, they are stable at 37°C. 2) Torpedo α subunit shows a 2- to 3-fold increase in binding to β-bungarotoxin upon shift from 37°C to 20°C.

419.7 ATP-ACTIVATED CHANNELS IN RAT AND FROG SENSORY NEURONS. B.P. Beno, C. A. Williams, and P. W. Cepko. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

External ATP (1-3 μM) induced an increase in conducibility in about 30% of bullfrog and 20% of rat DRG neurons tested. As reported by Krishtal et al. (Neurosci. Lett. 35:41,1983), the ATP-induced current was cation-selective, linearly-rectifying, and reversed near 0 mV. The induced current increased 3.5-4 fold for a doubling in [ATP], suggesting gATP ~ 1. The rapid onset of ATP-activated current (3-50 ms at 0.3 μM ATP, ~15 ms at 100 μM ATP) is nearly identical to that of a single channel blocker. The ATP-induced current is unlikely to be due to labile endogenous ATP or a general excitatory effect. Different channels in outside-out patches flickered rapidly (10s-100 mV) when activated, with a mean current of about 0.5 pA at -100 mV. The fraction of time a channel was in the activated, flickery state approached 1 at high [ATP]. Whole-cell noise showed exactly the properties expected if such channels underly the macroscopic current.


The recent cloning of the bovine brain GABA A receptor α and β subunits (Schofield et al., 1987) has led to the further isolation of two additional GABA A subunit types (β3, β2) (Levitan et al., submitted). The properties of these cloned subunits have been determined and compared with injectable synthetic RNA into Xenopus oocytes and studying the induced GABA-sensitive currents with voltage- and patch-clamp methods. Expression of any of the subunits together produces GABA-activated Cl - currents that are potentiated by the pentobarbital and inhibited by bicuculline and the 30-40 fold sensitivity of the Cl - channel to GABA is characteristic of multiple single-channel conductance states and voltage-dependent gating. A functional difference occurs when the α subunits are interchanged, the apparent sensitivity of the receptor for GABA is shifted 30-fold from 1.3 μM to 42 μM (E50). The order of sensitivity is α(4) β(3,2,1), with α 2 being most sensitive. The results suggest that functionally distinct subtypes of the GABA A receptor exist in the brain. However, these receptors are partially cooled since they lack benzodiazepine and are activated by GABA with a Hill coefficient of only 1. This suggests that additional subunits or processing is required for normal GABA A receptor function. Supported by NSF (ESI) and NIH (LACR, VEB).
419.9 SINGLE SUBUNITS OF THE GABAa RECEPTOR FORM ION CHANNELS WITH PROTEIN UTERILE CHARACTERISTICS OF THE N-LAMINAR GABAa RECEPTOR, I.A.C. Blair, E.S. Levitan, V.E. Dionne* and E.A. Barnard* MRC Molecular Neurobiology Unit, Cambridge, England.

Alpha and beta subunits of the GABAa receptor were expressed individually in Xenopus oocytes using RNA synthesized from cloned DNAs. GABA-sensitive, chloride-selective channels were detected several days after injection of one of these different RNAs, and with or without B RNA. The channels induced by each of the single subunits were indistinguishable. They had multiple conductance levels (nominally 10, 18, 28 and 44 pS), and their activity was potentiated by 20µM pentobarbital and inhibited by EGTA picrotoxin in which the sites for these agents are present on each subunit. Based on conservative subunit tests, the α and β subunits of the GABAa receptor show 55% amino acid sequence homology. The finding of each α and β subunits, examined separately, form GABA-sensitive ion channels with bacteriatoxin and picrotoxin regulatory sites and normal permeation properties suggests that the amino acid sequences which confer these properties in the native receptor are within the shared domains.

Supported by NIMH (LACB, VED) and NSF (ESL).


Characteristics of glycine-activated currents in 10 to 20 day old neurons were studied using the gigaseal whole-cell technique. Cells were voltage-clamped to -70 µV, and brief pulses of glycine were applied by pressure ejection from a second pipette containing 1 M glycine. Glycine produced inward currents that had a shift in reversal potential of -60 µV for a 10-fold reduction in external Cl- concentration, indicating that the alias were due to activation of a Cl' conductance.

Dose-response data were obtained for glycine in control and neurons calcium-containing different concentrations of strychnine (100 nM to 100 µM). Strychnine had mixed inhibitory actions on the glycine-induced currents that in both the neurons responses were decreased and the dose-response curves for glycine were shifted to the right. Glycine receptors with at least two different sensitivities to strychnine were found, based on the apparent inhibition constants (K_i). These two K_i values were associated with different Hill coefficients for glycine responses; i.e. K_i = 4.3 ± 0.9 x 10^(-3) M (± SEM, n=5), K_i = 1.8 ± 0.4; and K_i = 2.3 ± 0.8 x 10^(-3) M (± SEM, n=6), K_i = 3.8 ± 0.7. These differences were not related to culture conditions or age, and may reflect properties of multiple glycine receptor populations. (Supported by NSF #BBS 841780.)


We have examined the effects of two quaternary ammonium ions commonly used to block NMDA channels: tetaethyl- and tetrabutylammonium (TEA and TBA), on excitatory amino acid responses using patch clamp recording methods (whole-cell and outside-out patches) from mammalian central neurons in cell culture. Kainate (20µM) and AMPA (5µM) whole-cell currents, activated by either TEA (1mM) or TBA (1mM) in a voltage-dependent manner with a greater effect than -60V than at -40mV. Reduction of NMDA channel current amplitude by TBA was dose-dependent and qualitatively similar to the effects glutamate. TBA appeared to cause a flickering block without a noticeable change in conductance. Concentrations of Mg, Co, Ni and Mn ions, which might show similar effects, were found to be too low to account for the effects of TBA or TEA. Thus, the K-channel blockers TEA and TBA also block NMDA responses at similar concentrations as they affect K-channels. Detailed analysis of TBA currents will allow us to determine more about the mechanism of action of these blockers. Supported by NIH grant NS24467.

418.0 DEGENERATIVE DISEASE: OTHER I


Intrastriatal injections of quinoline (QUIN) can produce lesions which mimic many of the neurochemical and histopathological characteristics of Huntington's disease (HD). We studied levels of VIP and CCK by radioimmunoassay in chronic QUIN-injected Sprague-Dawley rats and in HD postmortem brain. Levels of VIP were elevated 3- to 4-fold in HD striatum, and 4- to 5-fold in QUIN-injected striatum. CCK was mostly normal in HD striatum as compared with a 2- to 3-fold increase in QUIN-injected rats. In cerebral cortex CCK immunoreactivity was elevated by 56-125% in all 13 areas that we examined, while VIP was increased to a lesser extent. There was no correlation between peptide levels and the degree of striatal atrophy. QUIN-injected rats had normal cortical concentrations of CCK and VIP.

These results suggest that (1) a chronic QUIN lesion in the rat can produce changes in striatal VIP immunoreactivity that are similar to those seen in HD, and (2) HD cerebral cortex contains increased levels of CCK immunoreactivity that are probably not adequately explained on the basis of cell loss and feedback from the striatal-pallidal-thalamic circuit.

420.2 PATCH MATRIX DISTRIBUTION OF CHOLECYSTOKININ AND CYTOKRINE OXIDASE ACTIVITY IN NORMAL AND HUNTINGTON'S DISEASE STRIATUM. JN. Ferrante, NW. Kowall and EP. Richardson Jr. Massa c h u s e t t s  G e n e r a l  H o s p ital and Harvard Medical School, Boston MA 02114.

The normal human striatum can be divided into two compartments based on the distribution of neurotransmitter projections. In Huntington's disease (HD), acetylcholinesterase (ACHE) histochemistry shows persistence of this patch matrix pattern despite marked atrophy and neuronal loss. We examined the distribution of cholecystokinin (CCK) and cytokrine oxidase (CO) in 6 HD brains and 6 age-matched controls to further characterize matrix relationships in HD striatum.

Clusters of CCK immunoreactive fiber terminals were heterogeneously distributed in both control and HD striatum. This patchy distribution is not different between normal and HD striatum. Comparison with adjacent ACHE stained sections showed that CCK distribution corresponded to low ACHE patches. The density of CCK patches was not altered in HD, and there was no evidence of CO positive matrix regions with negatively stained matrix increased.

In rodent striatum CO activity is patchy and enriched in spiny neuron dendrites. We found CO activity to be patchy in human striatum with regions of high CO activity corresponding to high ACHE matrix. In HD striatum CO activity was reduced, especially in the dorsal striatum. Thus extrinsic CCK immunoreactivity in normal striatal patches is preserved while striatal matrix enriched CO is depleted. These findings confirm the relative preservation of striatal patches and a subtle shift in systems and relative sensitivity of spiny neurons to destruction in HD.
420.5 VERBAL FLUENCY IN PROGRESSIVE SUPRANUCLEAR PALSY (PSP).
S. T. Smith* (SPON: W. A. Wilson). MGH Neurolinguistics Laboratory, Boston, MA 02118. Studies of cognitive processing in PSP commonly report severely impaired performance on tests of verbal fluency (production of words in a semantic or letter category in a specified time). Since these tests tap a variety of cognitive abilities (e.g., initiation, concept formation, abstract thinking, language ability and processing speed), breakdown in any of these abilities can cause impaired performance. In PSP, aspects of processing associated with frontotemporal dysfunction may be implicated.

In a case study of a 65 yr. old PSP patient with impaired fluency, an auditory recognition test designed to complement the fluency tests was administered. Experimental words were tested for inclusion in their letter and semantic category (e.g., pilot: F words; occupation). A subset of words was randomly selected from the complete structure of the 3-HAO enzyme, and to provide a critical reagent for examining the expression of this gene in the central nervous system. Supported by USPHS grants NS16102, NS20509 and NS16367.

420.4 DEGENERATIVE DISEASE: OTHER I


Studies in ALS have suggested an impaired production of RNA in surviving anterior horn cells (ahcs) and accumulation of neurofilaments (Nfs) in the proximal axons of ahcs. We evaluated the gene expression of the light neurofilament subunit (NF-L) in control spinal cords. Neuronal counts showed approximately 50% loss of ahcs in the ALS cases. The area of individual ahcs was decreased in the ALS case by approximately 25%. Total RNA recovery from the spinal cords was similar in ALS and control cases (p < .05). To date, RNase protection assays using an Nf-L riboprobe (JP Julien) have shown no difference in amount of NF-L mRNA in ALS as compared to control cases. Further, two animals in the Hyp + Ath showed disproportionate impairment in CNS function. The data suggest that hypertension alone, and perhaps to a greater extent when combined with an atherogenic diet, can produce marked impairment in CNS function. (Supported by Grant HL.13262)

420.7 THE EXPRESSION OF SPECIFIC PURKINJE CELL ANTIGENS IN TUMOR TISSUE FROM PATIENTS WITH PARANEOPLASTIC CEREBELLAR DEGENERATION. H.M. Furneaux*, D. Barbut*, P. Yee* and J.B. Posner. Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
Paraneoplastic cerebellar degeneration (PCD) is a rare but devastating disease in adults characterized by cerebellar symptoms in patients with an autoimmune etiology. The link between the generation of these autoantibodies and the occurrence of systemic cancer is not clear. We have now demonstrated by Western blot analysis (using human and rabbit antibodies) the expression of both the 67 and 34 kDa Purkinje cell antigens in tumor tissue from PCD patients. These results strongly support the hypothesis that paraneoplastic cerebellar degeneration is caused by an immune response to cerebellar Purkinje cell antigens provoked by their inappropriate expression in tumor tissue.


Quinoline (QUIN) is an endogenous brain metabolite which can cause selective excitotoxic neuronal death when introduced intraperitoneally in rats. The synthetic equivalent for QUIN, 3-hydroxyanthranilic acid oxidoreductase (3-HAO) has been purified from rat liver and its presence has been demonstrated in the mammalian brain. To further elucidate the biological role of 3-HAO in the brain, we have isolated a candidate cDNA clone for rat 3-HAO. Using a polyclonal antiserum raised against purified rat 3-HAO (J. Neurochem.49, 771, 1987), we screened a rat cDNA library constructed in the AGI expression vector. Two candidate 3-HAO cDNA clones, B1 and B2, were obtained. Each clone contained an approximately 1.5 kb insert which was subcloned into pUC19 for subsequent analyses. DNA sequencing revealed that the two were identical and contained only part of the putative coding region for 3-HAO. A search for DNA homology revealed the sequence to be unrelated to any previously reported genes. Efforts are under way to isolate additional cDNA to determine the complete structure of the 3-HAO enzyme, and to provide a critical reagent for examining the expression of this gene in the central nervous system. Supported by USPHS grants NS16102, NS20509 and NS16367.

420.8 A PRIMATE MODEL OF CEREBROVASCULAR DISEASE: BEHAVIORAL AND NEUROPATHOLOGICAL STUDIES. M.B. Moss, T. Kemper*, D.L. Rogers*, P. Prusis* and W. Hollander*. Boston University School of Medicine, Boston, MA 02118.
The behavioral and neuropathological consequences of hypertension and atherosclerosis were assessed in male cynomolgous monkeys that received coarctation of the thoracic aorta, alone (Group Hyp), or in combination with maintenance on an atherogenic diet (Group Hyp + Ath, N=5) for 12 months prior to testing. Post-treatment performance on a visual recognition memory task was compared to that of unoperated control animals maintained on an atherogenic diet (Group Ath, N=5) and unoperated control animals maintained on a normal diet (Group N, N=4). Performance by the Hyp group was significantly different from each other, was impaired relative to the N and Ath groups. Further, two animals in the Hyp + Ath showed disproportionate impairment. In contrast, the animals in Hyp alone did not differ significantly from Group N on the recognition task. Initial histological assessment revealed multifocal neuropathologies in monkeys in the Hyp + Ath and Hyp groups, including perivascular hemorrhage, mineral deposits, demyelination and ischemic infarction. The data suggest that hypertension alone, and perhaps to a greater extent when combined with an atherogenic diet, can produce marked impairment in CNS function.
A frequent complication of acquired immune deficiency syndrome (AIDS) is dementia. We evaluated 7 AIDS patients (age 28-55, all males) for evidence of AIDS dementia complex (ADC). The assessment included a psychiatric evaluation, neuropsychological(NP) testing, Computed tomography (CT), and 123-I-IMP SPECT. Six of the 7 patients exhibited cognitive (5 with memory loss) or behavioral (2 with new onset of inappropriate behaviors) abnormalities on psychiatric examination. NP testing showed general deficits, but none of the cases exhibited sufficient impairment to be classified as dementia. The SPECT scans showed marked asymmetry in the posterior temporal parietal region (posterior/ anterior = 1.17). This was concluded that type-2 AS and their precursors express two types of excitatory amino acid agonists tested did not appear to be related to stimulation of cGMP formation. In fact, the level of cGMP produced by 50 µM KA was largely prevented by an equimolar concentration of QA. However, 2 µM QA (ineffective by itself) generally potentiated the releasing action of KA. Cerebellar bipotential transmitter release, but not to the synthesis of cGMP. The releasing effect was activated by nitroprusside (200 µM) the level of cGMP increased 3- to 5-fold in CA2+ influx, 3-fold increase in Ca2+ efflux and a 5-fold increase in the release of noradrenaline (NE) from sympathetic neurons. The NMDA-stimulated Ca2+ influx and NE release were inhibited by the specific NMDA-receptor antagonist, D-2-amino-5-phosphopentanoate, showing receptor mediation. The activity of the polynucleotide (PA) synthesis regulating enzyme, ornithine decarboxylase (ODC), increased 2- to 3-fold within 15-20 sec of NEA exposure with a constant > 1.5-fold elevation in putrescine concentration. The specific ODC inhibitor, α-difluoromethylornithine (DFMO), blocked NMDA-stimulated Ca2+ influxes and NE release. DFMO inhibition was negated by the incorporation of putrescine in the incubation medium. Thus NMDA-controlled stimulation of synaptic transport functions appear to be dependent on PA which serve as messengers mediating cellular responses by enhancing Ca2+ fluxes needed for PA and Ca2+ dependent processes. (Supported by the VA and NIH grants RL 26835 and RS 18047)

EXCITATORY AMINO ACIDS IX

POLYAMINE DEPENDENCE OF NMDA RECEPTOR-MEDIATED Ca2+ FLUXES AND TRANSMITTER RELEASE FROM RAT HIPPOCAMAL SYNPATOSOMES. F. Siddiqui*, Z. Iqbal and H. Koennig. Neurology Dept., Northwestern University Medical School and Neurology Service, VA Lakeside Medical Center, Chicago, IL 60616.

The N-methyl-D-aspartate (NMDA) receptor is well characterized with respect to studies on excitatory effects of amino acids. In rat hippocampal synaptosomes, CA2+ influx is partially Ca2+-dependent in the case of KA. The releasing effect was activated by nitroprusside (200 µM) the level of cGMP increased 3- to 5-fold in Ca2+ influx, 3-fold increase in Ca2+ efflux and a 5-fold increase in the release of noradrenaline (NE) from sympathetic neurons. The NMDA-stimulated Ca2+ influxes and NE release were inhibited by the specific NMDA-receptor antagonist, D-2-amino-5-phosphopentanoate, showing receptor mediation. The activity of the polynucleotide (PA) synthesis regulating enzyme, ornithine decarboxylase (ODC), increased 2- to 3-fold within 15-20 sec of NEA exposure with a constant > 1.5-fold elevation in putrescine concentration. The specific ODC inhibitor, α-difluoromethylornithine (DFMO), blocked NMDA-stimulated Ca2+ influxes and NE release. DFMO inhibition was negated by the incorporation of putrescine in the incubation medium. Thus NMDA-controlled stimulation of synaptic transport functions appear to be dependent on PA which serve as messengers mediating cellular responses by enhancing Ca2+ fluxes needed for PA and Ca2+ dependent processes. (Supported by the VA and NIH grants RL 26835 and RS 18047)
421.5

STIMULATION OF INOSITOL PHOSPHOHYDROLYSIS BY EXCITATORY AMINO ACID receptor agonists added to gliial cells in primary culture increased ino- 
estriatal uptake of [3H]myo-inositol. Phosphohydrolase activity in the pre-
synaptic nerve terminals of the hippocampal formation, which increased ino- 
estriatal uptake of [3H]myo-inositol. Phosphohydrolase activity in the pre-
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421.6


The brain content of kynerenic acid (KYN), a tryptophan metabolite which acts as an antagonist at the excitatory amino acid receptor, was measured using an original method based on ion exchange chromatography and RPLC. Carli et al. Analyt. Biochem.169: 89-94, 1988. In newborn animals brain KYN content was extremely low (1.52 pmol/g protein, mean±SE), but an increase of two to three times occurred during the first 60 days of life. After sexual maturation brain KYN content continued to increase, reaching values of 74±16 pmol/g protein in 18 month old animals. Aging did not change KYN content in the liver or the kidney. The brain KYN content also significantly increased after the administration of tryptophan (50-500 mg/kg i.p.) or of its keto analogue: indolpyruvic acid (IPA 50-500mg/kg i.p.). Indirect evidence suggests that IPA may be directly metabolised to KYN that IPA administration may be considered as a new approach to antagonize "in vivo" excitatory amino acid receptors. Several pharmacological actions of IPA: sedation, analgesia and anticonvulsive effects, may be explained by its metabolization to KYN.

421.7


Excitatory amino acid receptor agonists added to gliial cells in primary culture increased inositol phosphohydrolase activity (IPH). Two to three times increase of inositol phosphohydrolase activity was measured using a original method based on ion exchange chromatography and RPLC. Carli et al. Analyt. Biochem.169: 89-94, 1988. In newborn animals brain KYN content was extremely low (1.52 pmol/g protein, mean±SE), but an increase of two to three times occurred during the first 60 days of life. After sexual maturation brain KYN content continued to increase, reaching values of 74±16 pmol/g protein in 18 month old animals. Aging did not change KYN content in the liver or the kidney. The brain KYN content also significantly increased after the administration of tryptophan (50-500 mg/kg i.p.) or of its keto analogue: indolpyruvic acid (IPA 50-500mg/kg i.p.). Indirect evidence suggests that IPA may be directly metabolised to KYN that IPA administration may be considered as a new approach to antagonize "in vivo" excitatory amino acid receptors. Several pharmacological actions of IPA: sedation, analgesia and anticonvulsive effects, may be explained by its metabolization to KYN.

421.8

FURTHER STUDIES WITH DEXTROMETHORPHAN AS AN N-METHYL-D- 

421.9

421.10

MULTISTITUTE MODEL OF NMETHYL-D-ASPARTATE RECEPTOR FUNCTIONING. D.C. Jent and S.R. Warf, Dept. of Psychiatry and Neurosciences, Albert Einstein College of Medicine, Bronx, N.Y.

Phenolcarboxylic (PCP) receptors have been shown to represent a site on the astrocyte cell membrane that interacts with the N-methyl-D-aspartate (NMDA) receptor complex. The interaction between PCP and NMDA receptors was studied using the selective PCP receptor ligands: [3H]D-(+)-5-Methyl-10,11-Dihydro-5H-dibenzo[a,d]cyclohept-5,10-imine maleate ([3H]DAM). Specific binding of [3H]DAM was stimulated by excitatory amino acids (EAAs) with equal potency to that of NMDA. Similarly, PCP receptors were not reduced by these drugs. Effects of dextromethorphan on PCP were much similar to that of ketamine.

In cortical slices in vitro, IC50 values for reduction of NMDA were: PCP 0.4uM, dextromethorphan 3.2uM, ketamine 19uM and dextromethorphan 19uM. Compounds such as carphen, n-caspine and phyton, ligands for a dextromethorphan/cannabinoid binding site (Nicchia et al., Neuropharmac., 26:999-1007) were active in vivo antagonists. With all four PCP-like drugs, development of the block of NMDA was agonist-dependent, a finding consistent with the view that they act at a similar site within the channel coupled to the NMDA receptor.
421.11 MAPPING OF A FROG BRAIN KAINIC ACID RECEPTOR WITH POLYCLYRAL ANTIBODIES. Westphal, R.I., Doehne, C.D., Hampson D.R., Wheaton*, K.D. and Obendorfer*, M.D. Laboratory of Neuro-otontology, NINCDS, NIH, Bethesda, MD 20892

We have produced a frog receptor from frog (Rana pipiens) brain using ion ion exchange chromatography and domoic acid affiniti chromography (Hampson and Westphal, J. Biol. Chem. 263-2500, 1988). The purified receptor showed high and low affinity components with dissociation constants and inhibition constants similar to those of the membrane-bound or crude soluble receptor. Polyclonal antibodies were made in rabbits with injections of 15-30 micromicros of the affinity purified preparation. Western analyses of antibody binding to whole frog brain showed several closely-spaced reactive bands which migrated with the purified receptor (Mr = 105,000). Analysis of frog liver, heart and muscle showed no corresponding immunoreactive bands. An antibody binding assay showed that the frog brain kainic acid receptor was strongly recognized by the antibody. However, the antibody did not react with kainic acid receptors from several other species.

For immunocytochemical analysis frog brains were fixed by immersion or frozen with 4% paraformaldehyde. Immunoreactivity was found throughout the brain with heaviest staining in the telencephalon and lowest in brain stem. The immunocytochemical analysis closely followed the distribution of the receptor determined autoradiographically using 3H-kainic acid bound to cryostat sections. At higher magnifications, kainic acid immunoreactivity showed a punctate labeling, often appearing to follow neuronal fibers. Cell soma labeling was not present.

421.21 SINGLE OR REPEATED MILD STRESS INCREASES SYNTHESIS AND RELEASE OF HYPOTHALAMIC-CORTICOTROPIN-RELEASING FACTOR (CRF). D.A. Haas and S.R. George, Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

Since CRF is believed to mediate the stress response in CRF, we examined the effect of a specific mild stress on CRF in hypothalamus of adult male rats. CRF immunoreactivity (CRF-ir) was determined using a rat CRF-specific RIA, and the relative contributions of synthesis and release. A single 5 minute restraint significantly increased CRF-ir in the median eminence 24 hrs later compared to controls (p<0.025), with no change detected thereafter. Plasma MONI was significantly elevated within 15 minutes of restraint (p<0.025). Repetition of a mild stress daily for 9 days resulted in significantly increased CRF-ir in hypothalamus 24 hrs later. This change could be due to either increased CRF synthesis or inhibition of release. In order to investigate the mechanism involved, the 5 min restraint was repeated while protein synthesis was blocked with the protein synthesis inhibitor anisomycin. This resulted in significantly decreased CRF-ir in hypothalamus 24 hrs later. These data show that mild stress increases net hypothalamic CRF content as a result of the balance between augmented synthesis and augmented release.

422.2 CHRONIC CORTICOTROPIN-RELEASING FACTOR (CRF) ALTERS RAT BRAIN BIogenic AMINE RECEPTOR BINDING. L.L. Gillen & J.G. Pettit, Department of Pharmacology, George Washington Medical Center, Washington, D.C. 20037

Since one of the prime mediators of the stress response is CRF, we examined the effect of a specific mild stress on CRF in hypothalamus of adult male rats. CRF immunoreactivity (CRF-ir) was determined using a rat CRF-specific RIA, and the relative contributions of synthesis and release. A single 5 minute restraint significantly increased CRF-ir in the median eminence 24 hrs later compared to controls (p<0.025), with no change detected thereafter. Plasma MONI was significantly elevated within 15 minutes of restraint (p<0.025). Repetition of a mild stress daily for 9 days resulted in significantly increased CRF-ir in hypothalamus 24 hrs later. This change could be due to either increased CRF synthesis or inhibition of release. In order to investigate the mechanism involved, the 5 min restraint was repeated while protein synthesis was blocked with the protein synthesis inhibitor anisomycin. This resulted in significantly decreased CRF-ir in hypothalamus 24 hrs later. These data show that mild stress increases net hypothalamic CRF content as a result of the balance between augmented synthesis and augmented release.


Rats pretreated with lactic acid showed a decrease in resting blood pressure (BP) a day after the stress of single social defeat (Fokkema, B.F. and Koelhaas, J.H., Physiol. Behav. 34: 530, 1985). The magnitude of this unexpected stress response has been investigated in male rats with experimental (DOCA/salt) and genetic (spontaneous, SH) hypertensive strains. Stress of social defeat on 3 consecutive days led to a substantial fall of resting BP of DOCA/salt and SH rats, and a slight increase in Wistar rats. This fall lasted the stress for a few days. Stress of forced swimming, shuttle-box avoidance and escape training, unavoidable footshock or condition avoidance of a single unaverted to a similar pattern of BP decrease in DOCA/salt hypertensive rats. Electrolytic destruction of the central nucleus of the amygdaloid complex, while administered antagonist before each exposure to stress enhanced the fall of BP. It is concluded that the inverse cardiovascular response of the amygdaloid complex is a protective role in its induction. (Supported in part by the Dutch Heart Foundation, grant no. 84.002.)


In work with separated infant rhesus monkeys tested in the presence of humans, we established that morphine at therapeutically efficacious doses produces less aggressive behavior than control rhesus monkeys (NEC), or in the presence of a human who maintained continuous eye contact (EC). A infants showed high levels of locomotion and DNA, in contrast with the infants in NEC decreased DNA levels (p<.002). Barking, an aggressive gesture occurring rarely in A or NEC, was evoked at high levels by EC (p<.002). Feeding and slow locomotion, rare in A and EC, increased in NEC (p<.002). We are now assessing the role of endogenous opiate, CRH, and benzodiazepine systems in mediating fear-induced aggression elicited by EC and feeding behavior elicited by NEC.
Depression is accompanied by disturbance of the HPA axis. These abnormalities have been shown to normalize with effective treatment including ECT. However, ECT effects are also seen in the KHT group since repeated seizures may act as a chronic stressor. Initial studies in patients compared the release of β-endorphin (βE) from the anterior pituitary with the first and last treatment. Overall, 11/17 individuals released more βE to the first treatment than the first. Those individuals who released less showed much shorter seizures with the final treatment than the first. To explore the mechanism involved in HPA changes with repeated ECT, studies were undertaken in rats. In the rat, there is also a greater release with treatment #8 than with treatment #1. Measurement of the anterior pituitary contents shows a 40% increase in βE in the chronic ECT rats. There is also an increase in CRF mRNA in the paraventricular nucleus of the hypothalamus. The adrenal weight is increased by 25%, and this result in a 3-fold increase in basal a.m. cortisol levels compared to unhandled control rats. There is also a shift in cortisol; in rats, the 1st treatment releases βE over β-LPH in a ratio of 2:1. This is also the case in man. However, the eighth session releases predominantly β-LPH with a ratio of β-LPH:βE of 2:1. In man, this shift in ratios does not appear to occur.

**SYMPATHETIC-ADRENAL MEDULLARY RESPONSE TO ACUTE FOOTSHOCK STRESS IN HYPERTENSION.** E.D. Hendley, M.A. Cierpi* and McAllen*, and B.M. Sharp* (SPON: F.J. Wilson), Mpls. Med. Ultrasound response to acute footshock stress. It had been by peripherally administered N. To localize the site of action WK-HAs and SHRs than in WK-HTs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with hypertensive trait that is also characteristic of SHRs. Two new inbred strains produced from a SHR X WK cross are WK-HAs, that are hyperactive but not hypertensive, and WK-HTs, that are hypertensive but not hyperactive. These two strains plus SHR and WKY were subjected to acute footshock. Plasma catecholamines increased significantly higher in WK-HTs than in WK-HAs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with the hypertensive trait, common to SHR and WKY strains, and not to the hypertensive trait. Supported by HL 29906, NS 00529 (R.M.) and Univ. Vermont College Medicine Research Committee (EM).


Four genetically related, inbred Wistar-Kyoto rat strains were used to examine the sympathetic-adrenal medullary response to acute footshock stress. It had been shown previously (McCarty & Kopin, Physiol & Behavior 21:567, 1978) that SHRs increase plasma catecholamines to higher levels than in WKY controls, in response to acute footshock. In this study we examined whether this sympathetic-adrenal medullary hyperreactivity is attributable to the hypertensive trait of SHRs or to the hyperactive trait that is also characteristic of SHRs. Two new inbred strains produced from a SHR X WK cross are WK-HAs, that are hyperactive but not hypertensive, and WK-HTs, that are hypertensive but not hyperactive. These two strains plus SHR and WKY were subjected to acute footshock. Plasma catecholamines increased significantly higher in WK-HTs than in WK-HAs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with the hypertensive trait, common to SHR and WKY strains, and not to the hypertensive trait. Supported by HL 29906, NS 00529 (R.M.) and Univ. Vermont College Medicine Research Committee (EM).

**HUMAN CATECHOLAMINE RESPONSES TO STRESS AFTER DEXAMETHASONE, SCOPOLAMINE PLUS AMPHETAMINE, AND PLACEBO.** R.L. Kohi. Universities Space Research Association, Space Biomedical Research Institute, Johnson Space Center, National Aeronautics and Space Administration, Houston, TX 77058.

Stress level was incrementally raised using a Staircase Profile Test (SPT) on a rotating chair assembly (Kohi, R.L. and Eponin, Med. 58:125, 1987). Subjects made head movements out of the plane of rotation until stimulation of the vestibular system induced averted nausea and multiple signs of a dynamic system dysfunction (ie, motion sickness). Dexamethasone (DEX) was loaded (3 mg/d) for 3 da and throughout a subsequent 5 da period of once daily SPTs. Scopolamine plus d-amphetamine (S/D, 0.4/5 mg) was administered 1.5 hr prior to 5 daily SPTs. Six blood samples were obtained prior to orally administered drug, and after drug on Monday and Friday, immediately before and after the SPT. Epinephrine (EPI) and norepinephrine (NE) levels generally rose following a SPT. DEX diminished or reversed the stress-induced rises in EPI and NE, particularly on Monday (p<.05). Poststress levels ranged from 25 to 65% of control. Lower pretest levels of NE in subjects receiving DEX indicated declining sympathoadrenal function (p<.05). S/D did not modulate catecholamine levels or responses to stressful motion. Because both drugs are effective, amnestic sickness drugs, it follows that reduction of peripheral sympathetic secretion probably does not underlie the therapeutic action of these agents.

**SYMPATHETIC-ADRENAL MEDULLARY RESPONSE TO ACUTE FOOTSHOCK STRESS IN HYPERTENSION.** E.D. Hendley, M.A. Cierpi* and McAllen*, and B.M. Sharp* (SPON: F.J. Wilson), Mpls. Med. Ultrasound response to acute footshock stress. It had been by peripherally administered N. To localize the site of action WK-HAs and SHRs than in WK-HTs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with hypertensive trait that is also characteristic of SHRs. Two new inbred strains produced from a SHR X WK cross are WK-HAs, that are hyperactive but not hypertensive, and WK-HTs, that are hypertensive but not hyperactive. These two strains plus SHR and WKY were subjected to acute footshock. Plasma catecholamines increased significantly higher in WK-HTs than in WK-HAs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with the hypertensive trait, common to SHR and WKY strains, and not to the hypertensive trait. Supported by HL 29906, NS 00529 (R.M.) and Univ. Vermont College Medicine Research Committee (EM).
HYPOTHALAMIC–PITUITARY–ADRENAL REGULATION I

422.11 IDENTIFICATION OF INTERLEUKIN-1 RECEPTORS IN MOUSE PITUITARY CELL MEMBRANES AND AT-T2 PITUITARY TUMOR CELLS. Daniel E. Frank*, Zouli E. Frank, and Erol B. De Souza (SPON: S. Frankin). Hypersensitivity Diseases Brain, Dept. of Physiology, University of Medicine, Brescia, Italy.

The cytokine interleukin-1 (IL-1) has a variety of effects on the brain, including stimulation of the hypothalamic-pituitary-adrenal axis. Whether IL-1 induces adrenocorticotropic hormone secretion by direct stimulation of cells in the pituitary or indirectly by the hypothalamic stimulation of corticotropic releasing factor is controversial. We examined this question by measuring the binding of [3H]-labeled recombinant human IL-1α (mIL-1α) to cell membranes of pituitary gland and brain regions. Specific binding of labeled IL-1α to pituitary membranes from C57BL/6 and other strains of mice was ten-fold higher than binding to Sprague-Dawley rat pituitary cell and adrenal gland cell membranes, indicating hypothalamic, and comparable to binding in mouse spleen. Labeled IL-1α bound specifically to whole cells or membranes from the AT-T2 mouse pituitary tumor cell line and the EL-4 mouse thymoma cell line. The binding of labeled IL-1α to mouse pituitary, AT-T2 and EL-4 membranes was temperature dependent, saturable, and of high affinity with a Kd of 40-60 μM and a Bmax of 8 (pitted) units (Bmax = 106 (pmol/mg protein) and 630,000 (pmol/mg protein) for AT-T2 and EL-4 cells, respectively). Labeled mIL-1α binding was significantly inhibited by mIL-1α, mIL-1β and an analog, mIL-1β (5α), in parallel with its relative bioactivities, but not by an inactive IL-1α peptide or by unlabelled peptides. These studies provide the first demonstration of IL-1 receptors in mouse pituitary and AT-T2 cell membranes with properties indistinguishable from the well-characterized EL-4 IL-1 receptors.


"Peripheral" benzodiazepine (pBZD) receptors are present in the pituitary, adrenal gland and testes. Although the role of these receptors in endocrine glands is unknown, pBZD receptor agonists modulate endocrine responses. Ro 5-4864, a pBZD receptor agonist, inhibits endoperoxidase secretion from AT-T2 cells, a mouse pituitary tumor cell line, and that the same agent increases basal and hCG-stimulated testosterone secretion in vivo. In the present study, we examined the involvement of the pBZD receptor in the secretion of immunoreactive ACTH (IR-ACTH) II) by stimulated rat hypothalamus. After exploration and overnight preincubation in medium 199 (M199), single hypothalamus were incubated in M199 for 40 min, followed by a 40 min stimulation with graded concentrations of Ro 5-4864 (Hoffman-LaRoche, Nutley, NJ) or Ro 5-4864 plus the pBZD receptor agonist PK 11195 (Dr. Le Fur, Pharmakus, France). The viability of each explant employed was tested, by examining the IR-ACTH response to 60 mM KCI-induced depolarization. Ro 5-4864 stimulated IR-ACTH secretion in a dose-dependent fashion (p<0.001, ANOVA followed by Duncan multiple range test) with ED50 of 3.5 ± 0.3 × 10−8 M. The stimulatory effect of 10−8 M Ro 5-4864 was completely antagonized by equimolar concentrations of PK 11195 (p<0.001, Student t test).

We conclude that activation of the pBZD receptors induces secretion of hypothalamic CRH in vivo. We speculate that the pBZD receptors may play a role in the in vivo regulation of the hypothalamic-pituitary-adrenal axis.

RECEPTOR MODULATION AND REGULATION III

423.1 DERIVATION TRIGGERS TRANSCRIPTIONAL ACTIVATION OF SKELETAL MUSCLE NUCLEAR RECEPTOR GLUCOCORTICOID RECEPTOR GENES. Michele Janney*, Denise M. Ewens*, and Jacob Schmidt (SPON: S. Yasuilla). Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook, New York 11794.

Transcriptional activity of acetylcholine receptor subunit genes were investigated in innervated and denervated chick skeletal muscle. The sciatic nerve of 3-day-old White Leghorn chicks was sectioned unilaterally, after various intervals, nuclei were isolated from operated and control (sham operated) animals and run-on assays performed. Nuclei were incubated with 32P-UTP, and total RNA extracted and hybridized onto filters containing an excess of subunit-specific DNA. Specific transcripts were detected by autoradiography and quantitated densitometrically. A sharp increase in transcriptional activity was observed which began approximately 12 h after the operation and peaked 1-5 days later when transcriptional rates reached approximately 5-6; and Sfoll control levels for the alpha, gamma, and delta subunit genes, respectively. A substantial decline, to less than 50% of control levels was seen by the fourth day after the operation. These results indicate that a denervation signal reaches the genome to induce receptor expression.


The present study investigates D-1 receptor plasticity using repeated treatment with serpine as an experimental model. Male SD rats were given serpine (1mg/Kg, i.p.) for 5 days and analyzed on the 6th day after the last injection. Striatal D-1 receptor subtypes were studied with 3H-SCH 23390 and by measuring gamma-Scatchard equation in response to the selective agonist SKF 82526. The responsiveness of adenylate cyclase (AC) to D-1 receptor stimulation was markedly induced after reserpine treatment (EC50 = 5.0mM in controls and EC50 = 0.6mM in serpine-treated rats); no significant changes were found in 3H-SCH 23390 binding density. In addition, formation of cAMP induced by 60nM was markedly enhanced in DA-depleted rats, while the responsiveness of AC to forskolin or to increasing concentrations of Mg-ATP was unchanged.


The effects of neurotensin in vitro were analyzed on the binding characteristics of 3H-3-propylnoradrenalepine (3H-NPA, a D-2 agonist in vitro) in membrane preparations of neostriatum and subcortical limbic areas (mainly nucleus accumbens and tuberoinfundibular lolution factor) in the rat. Under equilibrium conditions neurotensin increased the Vmax of 3H-NPA binding in a concentration related (0.3 – 100 nM) biphasic fashion with a peak increase at 3 to 122 ± 9 % in neostriatum and increases to 258 ± 30 % in subcortical limbic membranes. The Dmax-value of 3H-NPA binding was not significantly affected by neurotensin in the areas analyzed. Kinetic studies revealed that neurotensin increased the binding of 3H-NPA within 5 minutes of the addition of the peptide. These results indicate that the neurotensin receptor may be up-regulated by the presence of the neurotensin in vitro. The dose needed to induce maximal modulation of D-2 receptors is below the dose needed to saturate the neurotensin receptor. The ability of neurotensin to reduce the affinity of central D-2 receptors may be of importance for the etiology and treatment of schizophrenia and tardive dyskinesia.


Repeated administration of electroconvulsive shock as well as chronically treated with serpine increases alpha-1 adrenergic receptors as labeled by [3H]-Prazosin (Venelus, et al., Brain Res. 275:192, 1983; Stockepeter, et al., Eur J Pharmacol 192:259, 1989). Recent studies indicate that [3H]-Prazosin labels both alpha-la and alpha-lb subtypes with equal affinity, while [3H]-WB4101 labels primarily the alpha la subtype (Morrow and Creese, Mol. Pharm. 29:321,1986). To determine whether these subtypes could be regulated differentially by either serpine or ECS we treated rats for 10-12 days with ECS or for 15 days with serpine (1mg/Kg, i.p.) daily. Autoradiography confirmed homogenous binding studies in that ECS increased [3H]-Prazosin binding in most regions of the cortex by 18-46% and in the medial amygdala. Chronic treatment with serpine increased [3H]-Prazosin binding in the frontal cortex by 50% while [3H]-WB4101 binding was not changed by this treatment. Hence, in both homogenate and autoradiography studies, ECS appears to increase [3H]-Prazosin binding more than [3H]-WB4101 binding; while serpine, in homogenate studies, increased [3H]-Prazosin binding but not [3H]-WB4101 binding. These studies suggest that the two subtypes may be differentially regulated.
**423.5**

SELECTIVE REGULATION OF β₂-ADRENERGIC RECEPTORS BY DEAMINATED METHIONINE-VANADATE OR DEXAMETHASONE.

Glucocorticoids modulate the response of some tissues to β-adrenergic receptor agonists by increasing the density of β₂ receptors and the sensitivity of each subtype to glucocorticoids, (2) the presence of glucocorticoid receptors, or (3) the presence of other glucocorticoid-modulated proteins. Treatment of 16 myoblasts with 1 μM dexamethasone (DEX) increased the density of β₂ receptors by 9%, from 507 to 554 fmol/mg of protein. The effect of DEX treatment was dose-dependent, with an increase of 30% observed after treatment with 1 μM DEX. The DEX-induced receptor proliferation was maximal within 12 hr of the onset of treatment. The C₆ cell line is a glioma-derived line that expresses both subtypes of β receptors. Treatment of C₆ cells with 1 μM DEX for 16 hr selectively increased the density of β₂-adrenergic receptors from 25 to 37 fmol/mg of protein (50%). A smaller increase was observed after treatment with 10 nM DEX (31%). The DEX-induced proliferation of receptors was prevented by cycloheximide (100 μM). DEX had no effect on the density of β₁-adrenergic receptors. Thus, tissue-specific effects of DEX on β-adrenergic receptors may be due to receptor subtype specificity.

**423.7**


Opiate addiction could involve a change in the activity or binding of endogenous opioids. Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH₂), which can antagonize exogenous and endogenous opioids (Life Sci. 39:1153, 1986; Brain Res. 409:10-19, 1987), could play such a role. Tyr-MIF-1 can bind to some opiate receptors with low affinity and to morphine-labeled sites with high affinity, but its own binding sites do not have high affinity binding sites. These findings provide the evidence that µ-opioid receptor possesses two different coupling sites in brain tissues in vivo. The µ-receptor possesses two different coupling sites in brain tissues in vivo. The µ-receptor possesses two different coupling sites in brain tissues in vivo.

DIFFERENTIAL INTERACTIONS OF [{\textsuperscript{3}H}]2-OKO-QUAEPAX BINDING WITH THE GABA RECEPTOR COMPLEX IN SEVERAL RAT BRAIN REGIONS. B. J. Baldwin, D. J. Z. Miller, and S. B. Schuman.


REGENERATION: GENERAL II

1055

M.B.Graeber*, G.Raivich* and G.W.Kreutzberg

body) revealed a transient increase (days 2-14)

methylazoxymethanol were studied in no­

transferrin receptor expression were studied in no­

main advantages in using this system: first, we obtained

The distribution and time course of trans­

The processes of these cells project to the RCeG via

INCREASED TRANSFERRIN RECEPTOR EXPRESSION BY

binding sites confined to the regenerating nu­

tinsried, F.R.Germany.

PCR amplification and sequencing and for

INCREASED TRANSFERRIN RECEPTOR EXPRESSION BY

The absence of lamin in the central nervous system of adult mammals might be a reason for their poor regenerative ability. Previously, we have shown that medium conditioned (CM) by regenerating fish optic nerves, applied in situ to injured optic nerves of adult rabbit, causes an increased appearance of lamin immunoreactive sites (Zak et al. Brain Res. 408: 263-1987). This observation suggests that CM can modulate lamin levels in gial cells. In the present study, we show that this CM can activate C6-glia cells to synthesize and accumulate lamin. The level of lamin immunoreactive sites was elevated in cells treated with CM. The CM-induced effect could be detected either by ELISA screening method for surface antigen or by immunofluorescence, using lamin specific antibodies. The optimal effect was observed at concentrations of 0.1 ug protein/ml, even higher than the effect of 10% fetal calf serum. The identity of the lamin immunoreactive protein and the observed effect of CM on C6-glia cells was verified by metabolic labeling of these cells followed by immunoprecipitation and gel-electrophoresis. The CM-induced effect was not unique to lamin as a similar elevation could be observed in fibrinectin level (another matrix protein). The CM-induced effect could be detected in the pool of high salt extractable proteins as well as in the medium. Basing of the CM completely abolished its activity, suggesting that a proteinaceous component(s) within the CM is responsible for the activation. Production of lamin may be a necessary step in the induction of regeneration. Therefore, application to injured mammalian CNS, of factor(s) originating from regenerating systems, that modulate lamin production or accumulation in gial cells may circumvent one of the impediments to regeneration.

CONDITIONED MEDIA OF REGENERATING FISH OPTIC NERVES MEDIATE

LAMININ LEVELS IN GLIAL CELLS. Cohen A. and Schwartz M. The Weizmann Institute of Science, Dept. of Neurobiology.

The processes of these cells project to the RCeG via the right cerebropedal connectives, exit the RCeG via the penial nerve, and then reach the penial complex in the periphery.

The processes of these cells project to the RCeG via the right cerebropedal connectives, exit the RCeG via the penial nerve, and then reach the penial complex in the periphery.

Regrowth of axons in the central nervous system (CNS) may be limited by the absence of supporting nerve growth and/or the presence of inhibitory molecules found in CNS myelin (Garoni & Schwab, Neurosci. Res. 185:1988). Growth of dorsal root ganglion (DRG) cells and dissociated cells was assessed on culture substrates taken from the CNS (optic nerve) and PNS (facial nerve), of wild type C57/BL6 mice and shi/shi (an outbred stock). Shiverer is a hypomyelinated mutant lacking proteolipid and basic myelin proteins. Cytocat sections of optic and sciatric nerves 6mm thick were placed on poly-l-lysine coated substrates, cultures of DRG explants and dissociated cells were prepared from E19 mouse embryos, plated onto the tissue substrates, maintained in DME/F12 medium and examined for attachment and spreading. Shiverer optic nerve explants and dissociated cells did not grow and showed limited growth (up to 3X cell diameter). Shiverer optic nerve explants and dissociated cells did not grow and showed limited growth (up to 3X cell diameter). Shiverer optic nerve explants and dissociated cells did not grow and showed limited growth (up to 3X cell diameter). Shiverer optic nerve explants and dissociated cells did not grow and showed limited growth (up to 3X cell diameter). Shiverer optic nerve explants and dissociated cells did not grow and showed limited growth (up to 3X cell diameter).
425.1
HOMOGRRAFTED FETAL RAT CORTICAL ASTROCYTES MIGRATE FROM CORTEX INTO IMPLANTATION POCKETS THROUGHOUT ADULT HOST RAT BRAIN W.J. Goldberg and J.J. Bernstein. Laboratory of CNS Injury and Regeneration, VA Medical Center, Department of Neurobiology and Physiology, George Washington University School Medicine, Washington, DC.

The cerebral cortices from 14 day gestation rat embryos were prebleached with 1% Phaëbus vulgaris leucoagglutinin (PHA-L) and homografted into freshly made implantation pockets in host cerebral cortex. Animals were used 30 and 60 days later. Paraffin sections were stained with PHA-L specific antibody (GFAP, astrocyte specific marker), and PHAL (gial specific marker). PHA-L-GFAP positive cell was a graft derived astrocyte. Graft derived astrocytes (found on glia limitans, spaces of Virchow-Robin, migrated along pial and subependymal layers of the third and lateral ventricles. Graft derived astrocytes migrated ventrally through the gray matter at the base of the implantation pocket to enter the corpus callosum. The migration routes continued through intersecting nerve fiber bundles. Basal lamina (blood vessels, glia limitans, space between ependymal and subependymal layer) was another preferred migration route. Since a major component of basal lamina is laminin, these data suggest that laminin may be one of the cell surface recognition molecules for fetal astrocyte migration. Supported by the Veterans Administration.

425.3
THE MOLECULAR FORMS OF PLASMINOGEN ACTIVATOR IN DIFFERENTIATING ASTROGLIA ARE DEVELOPMENTALLY REGULATED N. Kaldarol. The Rockefeller Univ., New York, NY 10021

Flasminogen activator (PA) is the key enzyme which initiates a cascade of extracellular proteolytic activities. Mammalian cells produce two distinct molecular forms of PA, the tissue-type -- t-PA and the urokinase-type -- u-PA. Recent studies show that the role of u-PA is in control of cell migration/invasion, while t-PA is involved primarily in fibrinolysis. It was established that the cellular PA activity is 20-30 fold higher in astrocyte and developmentally regulated (Kaldarol et al., 1988 in Cur. Issues in Neural Regen. Res., Reiter et al., eds., in press). This study focuses on the characterization of the molecular forms of PA which are produced by differentiating rat astroglia in cell culture. It was found that: 1) immature glial cell cultures (the day 5 of 22 cells). The fourth and final class of channel is a high potassium current. This high potassium current has a larger amplitude and a very long open duration (10-50 ms) for varying lengths of time (15 sec to 30 min). Up- takes were terminated by 5 washes with 0.32 M (0-3 sec) uptake media containing Ca++. Uptake in astrocytes is energy dependent and reached a peak steady-state level of 6 to 7 nmol/mg protein within 10 min. No increase in the 90-sec uptake was observed when sodium was removed from the uptake media containing Ca++. Uptake in astrocytes was dependent on the presence of Na+ or Ca++ in the uptake media, and an outward channel current became observable at membrane potentials (-40 mV) to 0 mV. The channel is blocked by 1 m M extracellular potassium.

425.5
A STRETCH-ACTIVATED ION CHANNEL IN RAT ASTROCYTES IN PRIMARY CELL CULTURE. J.D. Dent*, J.Yang*, C.C. Bowman*, and F. Sachs*. (SPON: A. Auerbach). Department of Biophysical Sciences, SUNY at Buffalo, Buffalo, NY 14214

Using single-channel patch-clamp recording technique in the cell-attached configuration, we are investigating several channels that exist in astrocytes (a type of glial cell) derived from newborn rat brains. With a Mg-free KCl solution in both the pipette and the bath, we observe several classes of channels. The first and most common (15 out of 22 cells) class consists of brief events lasting several msec composed of several amplitudes. At depolarizing membrane potentials, the current and amplitude increased (suggesting that this class of channel is either Cl or K selective). The second class of channel has a larger amplitude and a very long open duration (seconds) (9 out of 22 cells). The third class of channel consists of spiky brief events, that occur in bursts at hyperpolarizing membrane potentials (5 out of 22 cells). The fourth and final channel of class is a slow gating (10 of msc) small amplitude event (20 of 22 cells).

With a 200 micromolar glutamate Kinger's solution in the pipette, the frequency of appearance of the slow gating channel (8 of 16 cells) and the spiky bursts events become observable at -60 mV, the normal membrane potential (5 of 16 cells). The reversal potential of the latter class of channel is near 0 mV.

We thank Drs. Malcolm Brodbeck and Mike Curren for useful suggestions. Supported by NIH grant NS23064 and a grant from CLF, NEI, to D.C.B., and USPHS DK 37792 and USARMD 2260-L to Frederick Sachs.

425.6
EFFECT OF NIMODIPINE ON POTASSIUM (K+) STIMULATED CALCIUM (((Ca++))) UPTAKE IN ASTROCYTES. H.S. White*, A.S. Bender*, E. Butler*, D.M. Woodbury, and L. Hertz*. Dep't of Pharmacol. and Tox., Univ. of Utah, S.L.C., UT 84112 and Dep't of Pharma. Col., University of Saskatchewan, Saskatoon, Canada S7N 0W6

Previous attempts to demonstrate that K+ stimulates 45Ca++ uptake into astrocytes have been unsuccessful. Primary astrocyte cultures were incubated with serum-free media containing 45Ca++ (0.5 Ci/ml) and K+ (5 to 120 mM) for varying lengths of time (15 sec to 30 min). Uptake was terminated by 5 washes with 0.32 M (0-3 sec) sucrose containing 2.0 mM EDTA. 45Ca++ uptake was time-dependent and reached a peak steady-state level of 6 to 7 nmol/mg protein within 10 min. No increase in the 90-sec uptake was observed when sodium was removed from the uptake media containing Ca++. 45Ca++ uptake into cells first loaded with 5 mM Ca++ for 60 sec and then depolarized with depolarizer and 45Ca++ uptake into primary astrocytes is enhanced by K+. 45Ca++ uptake into primary astrocytes is enhanced by K+. K+ stimulated the Ca++ channel blocker nifedipine. High concentrations (0.1 to 100 mM) of nifedipine partially reduced basal uptake. Thus, under optimal conditions, 45Ca++ uptake into primary astrocytes is enhanced by K+. Nifedipine blocks this effect suggests that the utility of Ca++ blockers in ischemia and epilepsy may be related in part to an effect on astrocytes. (Supported by NIH Grant R01-NS 22200 and MRC Grant MT5957).

Within the CNS, astrocytes play an important role in the regulation of extracellular K+. The mechanisms of this potassium uptake and spatial buffering. The present investigation was initiated in order to assess the effect of phorbol esters on active K+ uptake into astrocytes in gliotoxic astrocytes in the mouse cerebral cortical astrocytes according to the method of Hertz (Ann. N.Y. Acad. Sci. 481:318-333, 1986). The active phorbol ester 12-myristate 13 acetate (PMA) inhibited both active uptake of 42K+ and passive permeability. Its effect on passive permeability was, however, significantly greater than its effect on active transport (IC50: ~0.07 vs 81 μM). In contrast, the inactive congener 4-phorbol 12,13-diacetate did not exert any effect on K+ permeability at concentrations up to 1 μM. Since it is widely recognized that phorbol esters activate PKC, the present results suggest that this enzyme plays an important role in modulating passive K+ permeability into astrocytes. This is a novel mechanism whereby passive K+ influx into astrocytes is regulated and may underly the pleiotropic effects of CNS excitability. (Supported by NIH Grant I-R01-22000 and MRC Grant MT5957).

425.9 IDENTIFICATION OF GLUCOCORTICOID REGULATED PROTEINS IN PURIFIED RAT CEREBRAL ASTROCYTES BY QUANTITATIVE 2D-GEL ELECTROPHORESIS. M.C. Bohn, A. Walenta*, M. Lynch* and J. deVellis Department of Neurobiology and Anatomy, Univ. of Rochester Medical Ctr., Rochester, NY 14642 and Mental Retardation Research Ctr. Univ. of California, Los Angeles, CA 90024.

Although glucocorticoids are known to act in target cells to regulate gene expression at the transcriptional level, only a few glucocorticoid regulated genes have been identified in the nervous system. Previous studies have demonstrated that glutamine synthetase is regulated at the transcriptional level in astrocytes. This study was undertaken to identify other glucocorticoid regulated proteins in astrocytes. Astrocytes were prepared from newborn rat cerebral cerebromotor cortex and grown in DMEM-P10 with 10% fetal calf serum. After 2 weeks, monolayers were removed by shaking, and the astrocytes replated and grown to confluence. On day 37, the cells were shifted to serum-free medium and, a day later, 10μM hydrocortisone (HC) added for 48 hours. During the last 16 hours, cells were labeled with 35S-methionine. Cells were then harvested onto nitrocellulose and subjected to 2-D gel analysis. Approximately 15% of the proteins were significantly increased or decreased by HC, suggesting that protein metabolism pathways are markedly affected by glucocorticoids. These data promise to lead to the identification of glucocorticoid regulated genes in the brain.

425.10 ASTROCYTES AS A SOURCE OF PROSTANOIDS IN THE CNS: RELEASE OF TRANSMEMBRANE EVOKED TRYPANBLUE. P.G. Galloway, M.J. Likave*, G. Perry, Case Western Reserve University, Cleveland, Ohio 44106.

Within the CNS, astrocytes can synthesise and release thromboxane (TX) and prostaglandins (PG). Using primary cultures of cells from neonatal rat brain we have shown that astrocytes can synthesise and release thromboxane (TX) and prostaglandins PGF2α and PGD2 (Murphy, S. et al., Neuroreport, 6:1-6, 1995). Activation of P2-purinergic receptors on vesicles by ATP prompts the release of prostanooids. In our astrocytes, ATP and ADP (EC50:50μM), but not AMP or adenosine, evoke TX release within 1 min, and this involves the activation of phospholipase A2 (PLA2). This action of ATP and ADP is mediated by P2 receptors linked to the hydrolysis of inositol phospholipids and the mobilisation of intracellular calcium. When cells are calcium-depleted, calcium ions are then stimulated with ATP, there is a marked delay in TX release associated with the time taken for the calcium pool to re-fill. This work is in collaboration with James Jeremy and Peresh Dandona (Royal Free Hospital, London), and is supported by a project grant from the SERC. The new permanent address for SM: Pharmacology, Unlv. Iowa, Iowa City IAA24242.


The biology underlying the formation of demyelinated plaques in multiple sclerosis (MS) is not well understood. Recent studies have suggested the involvement of an autoimmune process. The neuronopathic, substance P, has been implicated in the inflammation of autoimmune animal models. Substance P immunoreactive of cerebellar vessels and perivascular substance P immunoreactive astrocytes have been observed in normal tissue (Michel et al., Brain Res. 377:383, 1986). Using immunoperoxidase methods with well characterized rabbit polyclonal substance P (BP2C7), we examined human post-mortem brain tissue for substance P immunoreactive and MBP plaques. Preabsorbed antisera did not produce specific staining. Prominently stained astrocytes were found near the edge of the MS plaque often in association with small blood vessels. Many of the astrocytes were binucleate. Fewer substance P immunoreactive astrocytes were observed in the remainder of the plaque or surrounding tissue. These data suggest involvement of substance P immunoreactive astrocytes in the genesis of MS plaques.

425.12 TROPMYOSIN ISOFORM EXPRESSION IN NORMAL AND NEOPLASTIC ASTROCYTES. F.G. Galloway, M.J. Likave*, G. Perry, Case Western Reserve University, Cleveland, OH 44106.

Tropomyosin is a protein associated with microfilaments of multinucleate cells, having molecular weights from 29kD to 40kD. Changes in the expression of tropomyosin isoforms have been reported in transformed cells (J. Biol. Chem. 258: 1983). We have examined tropomyosin isoform expression in human astrocytomas by immunostaining brain and paper blots from brain tumor homogenates subjected to SDS-PAGE. Controls were normal human and rat fibroblasts. Both mouse and rat tropomyosin isoforms were used as follows by tissue of origin and isoform recognition: platelet, 20, 32kD (PT); brain, 30, 35kD (BT); smooth muscle, 32, 36kD (SM); and skeletal muscle 32 and 32kD (SM). Immunoreactivity of astrocytes in normal brain was present, but less intense than in neoplastic cells regardless of antibody. Tropomyosin isoforms using PT was stronger than for PT for all degrees of anaplasia of tumors. This suggests that the 35kD isoform may be synthesized to a greater extent than the 32kD isoform in neoplastic cells. The immunoreactivity with PT and SM was maximal in the cytoplasm, whereas with BT and SM it was in cytoplasm and processes. Cytologic staining by PT was small, but with BT it was granular. These data suggest that the different isoforms may have specific subcellular localization and organization in neoplastic cells. (Supported by the NCI Center Grant #P20CA43703 and CCHF Grant to PG.)
Control of Tyrosine Hydroxylase in Sympathetic Ganglia by Testosterone. M.E. Goldstein, A.W. Tank and R.W. Hamilton. Dept. of Neurology, University of Rochester/Monroe Community Hospital, Rochester, NY 14603.

The hypothalamic ganglion (HG) of the rat sympathetic nervous system is dependent upon the continued presence of testosterone for normal development and maintenance of tyrosine hydroxylase (TH) activity. Recent studies have shown that the decrease in TH activity in HG of adult male rats 1, 2 and 4 weeks following castration, and replacement of testosterone immediately following castration prevents this decrease. The regulation of TH by testosterone has been examined further to determine whether changes in TH activity are a direct result of decreased mRNA and protein levels or a change in the level of activity of preexisting enzyme molecules. Dot blots of total RNA isolated from HG hybridized with a cDNA probe for TH do not exhibit a decrease in HG. However, the decrease in HG TH activity is likely to be due to the direct effect of testosterone on TH activity. The results may have impact on the behavioral and psychological changes associated with pregnancy and post-partum period.


The pineal, via its hormone melatonin, is the primary transducer of photic information for neuroendocrine regulation of seasonal breeding. The site of melatonin action in the brain is uncertain; however, studies in mice have implicated the anterior hypothalamus (AH). The present study was undertaken to examine this site in the gerbil using double immunostaining techniques. Single- and double immunostaining techniques were employed in the hypothalamus of overnighted (OVX) and OVX - estrogen primed (estradiol valerate, EV; 4 days, s.c.) gerbils. Immunostaining for melatonin receptors (MCR), TH and SHT. Results: A dense population of PR immunoreactive neurons was observed in the hypothalamic infundibulum and ventromedial (VM) nucleus, and hypothalamo-hypophysial (HP) tract between the VM nucleus and the fornix. PR immunostaining was confined to the cell nucleus and increased markedly in intensity after EV treatment. Staining was unaffected by injection of progesterone (4mg, s.c. 2h before sacrifice). In colchicine pretreated monkeys (5mg, iv 48h prior to sacrifice), some perikarya of all PR immunoreactive neurons were immunoreactive for GABA, catecholamines, opiooids and 5-HT. (Supported by NIH grant No. HD33587 and St. Kitts Biomed. Res. Foundation).


The GABA-benzodiazepine-chloride ionophore (GABA-A) receptor complex in the brain is regulated by several endoprotease steroids, including tetrahydroprogesterone (THP). (Majewski, K. D., Biochem. Pharmacol. 36:1781 1987). Pregnancy is associated with greatly elevated levels of progesterone, whose metabolites may affect the function of the CNS.

We compared the binding of the GABA-A agonist, [3H]muscimol, in vitro, to tissue from non-pregnant rats and those at various stages of pregnancy. At 10 nM ligand concentration, there was about 50% and 30% more muscimol binding on days 15 and 19 of pregnancy, respectively, as compared to non-pregnant rats. This effect was due to a reduction of the KD of low affinity GABA-A receptor sites. The alterations of muscimol binding caused by pregnancy were similar to the effects of THP, in vitro, and followed the pattern of changes of progesterone levels in the plasma of pregnant rats. It is likely that these pregnancy-induced changes resulted from increased levels of GABA-A-agonistic progesterone metabolites in the CNS. The results may have impact on the behavioral and psychological changes associated with pregnancy and post-partum period.


Immunochemistry performed on 80um unembalmed tissue sections was used to study the distribution of GnRH neurons and fibers in the basal forebrain and amygdala of 2 adult female human brains. Sections were subjected to computer-assisted image analysis to generate a three-dimensional map of GnRH cell bodies. Cell bodies were concentrated in the preoptic area and basal hypothalamus, but were also evident in the suprachaoptic nuclei, septal region, anterior hypothalamic area, and cortical and medial amygdaloid nucleus. GnRH-containing fibers were observed within the hypothalamus (predominantly infralimbic region and lamina terminals), septum, striatum terminalis, olfactory tubercle, dorsal preoptic area, prefrontal cortex, lateral hypothalamus and medial and lateral orbitofrontal areas. Many fibers could also be seen coursing along the base of the brain between the hypothalamus and cortical and medial amygdaloid nuclei. The localization of GnRH-containing cells and fibers in several of these areas represents new observations in the human brain, and suggests an important role for the amygdala in the regulation of gonadotropin secretion in man. (NIA:1K11AG00295)
426.7 MECHANISM OF ACUTE LH-SECRETORY RESPONSE TO GnRH. J.L. Hug and M.E. Freeman*. Dept. of Neurology and Behavior, SUNY at Stony Brook, Stony Brook N.Y. 11794.

To further characterize the role of glucocorticoids in the regulation of LH secretion, we examined the effect of glucocorticoids on CRF mRNA observed following adrenalectomy (ADX) and its alteration by corticosterone (CORT) is being measured over time. Previously, CRF mRNA has been shown to increase in the rat hypothalamus after 7 days of ADX (Jingami et al., Endocrinology 131:141, 1990), specifically in the paraventricular nucleus (Young et al., Neurosci. Letts 70:198, 1988; Kovacs et al., Neuroendocrina, 48:385, 1987). Here, hypothalamic CRF mRNA levels change in response to glucocorticoids. The effect of CORT on CRF mRNA levels was measured by counting hybridizing bands on Northern blots from individual rats is hybridized to a rat CRF probe generated by a cDNA clone (gift of Michael D. Brownstein, National Institute of Mental Health, Bethesda, MD). The analysis of the data was performed using the NIH Image program.

CRF mRNA was observed to increase in the hypothalamus after 7 days of ADX and decrease in CRF mRNA levels in the females after 60' of CORT treatment and remained decreased at 60' and 4hr after CORT treatment. For long term studies, rats were treated with CORT and sacrificed after 30', 60' and 4hr for acute studies. For long term studies, rats were treated with CORT and sacrificed after 30', 60' and 4hr for acute studies.

In dispersed pituitary cells, studies of LH release in response to GnRH with 2 pulses of LH release within 30 min. Inhibition of protein synthesis by cycloheximide (1 mM, 30 min) did not interfere with either phase of short term LH release. Release of LH by depolarization with KCl (59 mM) started after only about 17 min of exposure. Osteoblasts of callus bone by EDTA(4 mM) did not interfere with either pulse of GnRH-stimulated LH release. In summary, dispersed pituitary cells responded to the challenge of GnRH with 2 pulses of LH release within 30 min. Thereafter, LH release increased linearly up to 4 hours. Thus the cytoskeleton is involved in short term release, calcium ions are not required.


Clonidine (CLON), an α2 adrenergic agonist, has been shown to stimulate GH secretion by releasing endogenous GRF, but the precise mechanism has been unclear. Since β-endorphin stimulates GH secretion by increasing GRF release, we explored its role in GH secretion. B-endorphin stimulates GH secretion by increasing GRF release, we explored its role in GH secretion. B-endorphin anisomycin (β-endorphin) as well as normal rat serum (NSM) and ACTH anisomycin (ACTH-NSM) as controls were examined in dispersed pituitary cells (β-NSM=1 μg/kg and β-NSM=1 μg/kg for 1 h) and 7 days after ADX. Four groups of rats were being studied: normal, sham ADX + placebo, ADX + placebo, and ADX + corticosterone (CORT, 25 mg, s.c.) at 9:00 am. Serum CORT levels, weight changes and thymus weights are being measured over time. Previously, CRF RNA has been shown to increase following acute CRF injection. In contrast to control levels, beta endorphin treatment caused an increase in CRF RNA observed following acute treatment with CRF. In contrast to control levels, beta endorphin treatment caused an increase in CRF RNA observed following acute treatment with CRF.

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426.9 KINETICS OF CRF mRNA LEVEL INCREASES AFTER ADRENALECTOMY. E.M. Lut and J.L. Hug., Dept. of Neurology and Behavior, SUNY at Stony Brook, Stony Brook N.Y. 11794.

Although chronic treatment with CRF raises the level of anterior lobe (AL) POMC mRNA and/or increased degradation of mature m RNA into the perifusate, or by cell depolarization with 50 mM K+. Thus, the secondary (plateau) phase appeared to be necessary for refilling intracellular Ca++ stores depleted during the initial (rise) phase.

These data are consistent with a mechanism by which CRF induces an initial rise in Ca++ via [Ca++]-mediated mobilization of intracellular Ca++ stores followed by a sustained [Ca++]i rise due to depolarization/depolarization Ca++-activated channel activity of VSCCs.


Growth hormone releasing hormone (GHRF) and somatostatin (SRIF) are two neurohormones which directly control growth hormone secretion. In an attempt to define the relationship between GRF receptors and GRF mRNA in the hypothalamus, we have compared the radiographic distribution of specifically labeled SRIF binding sites with the immunohistochemical distribution of GRF-containing neurons in the same region of the hypothalamus. SRIF binding sites were labeled in vitro using 125I- or 125I-SRIF. The distribution of these SRIF binding sites was similar to that of GRF-containing neurons throughout the ventromedial nucleus of the hypothalamus.

Conclusion: These results suggest that α2 adrenergic activation stimulates GRF secretion, at least in part, via β-endorphin as a mediator.

426.8 MECHANISM OF ACTION OF GONADOTROPIN-RELEASING HORMONE (GnRH) AS TESTED BY CULTURED CALCIUM CALCIMETER CONTAINING NEURONS IN THE RAT ARCULATE NUCLEUS. G. A. Shugart*, S. A. W. Murphy*, and R. J. Miller (Sidney Kimmel Cancer Center, Philadelphia, PA 19104)

Regulation of gonadotrophin secretion by GnRH has been shown to involve calcium ion (Ca++) influx through voltage-sensitive calcium channels (VSCCs). To explore the mechanisms by which Ca++ participates in this stimulus-secretion coupling, we studied Ca++ signals in single gonadotrophs from 35-day-old female rats, placed on ectodermal grafts, respectively identified via a reverse hemolytic plaque assay for luteinizing hormone (LH), and loaded with the Ca++ sensitive fluorescent indicator FURA-2, with a microperfusion/flush perfusion system. Pretreatment of cells with GnRH (100 μM) elicited an initial brisk rise in [Ca++]i, which then returned toward basal levels (~100-150 nM), only to rise again to a lower and more sustained secondary plateau phase, which preceded, and then returned toward basal levels (~100-150 nM). This is indicative of [Ca++]i levels through VSCCs, which could be brought about either by the mobilization of endogenous Ca++ stores by GnRH into the perifusate, or by cell depolarization with 50 mM K+. Thus, the secondary (plateau) phase appeared to be necessary for refilling intracellular Ca++ stores depleted during the initial (rise) phase.

These data are consistent with a mechanism by which CRF induces an initial rise in Ca++ via [Ca++]-mediated mobilization of intracellular Ca++ stores followed by a sustained [Ca++]i rise due to depolarization/depolarization Ca++-activated channel activity of VSCCs.

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Previous work has demonstrated that the in vitro neonatal rat brainstem-spinal cord is capable of generating rhythmic fictive locomotory activity similar to motor output patterns recorded during locomotion in preparations with intact limbs (Smith et al, The FASEB J 2:2283-88,1988). The in vitro model allowed us to explore neurochemical events underlying spinal locomotor systems which would not otherwise be feasible in vivo. In order to further investigate the validity of this preparation, the effects of spinal cord manipulations on fictive locomotion were obtained.

Neurons with discharge patterns of respiratory motor patterns of brainstem origin are known to be convergent excitatory or inhibitory inputs from several spinal motor output; (ii) neurons with spike discharge that brackets the I phase or with continuous E phase discharge. Intracellular recordings show 5-10 mV rhythmic membrane potential oscillations underlying spinal discharge in E neurons with temporal characteristics that in part account for the neuronal discharge patterns. Studies in progress are attempting to establish roles of the identified neuron populations in respiratory rhythmogenesis and motor burst pattern generation. Supported by NIH grant NS-22975 and the Herzel Trust.


Rhythmic motor patterns for locomotion can be generated by chemical or sensory activation of neonatal spinal cord in vitro (Smith et al, The FASEB J 2:2283,1988). In order to more precisely characterize the locomotor patterns in vitro, the activation patterns of select flexor and extensor hindlimb muscles were determined from electromyographic recordings. Fine wire electrodes were inserted in the tibialis anterior (TA), lateral gastrocnemius (LG), vastus lateralis (VL), and rectus femoris (RF) of one hindlimb in spinal cord preparations retaining innervated hindlimbs in vitro. The spinal cord circuitry was activated by dopamine, N-methyl-D-aspartic acid, or L-aspartyl glutaminyl acid uptake inhibitor dihydroxyacid. Chemically-induced activation patterns were compared to those generated by electrical activation (stimulus train). Comparisons were also made between in vitro locomotor patterns and those during treadmill locomotion in adult rats. The motor patterns in vitro consisted of multi-joint limb movements in which flexor and extensor muscles were reciprocally activated. The temporal relationships among the TA, LG, VL, and RF were similar to those recorded during treadmill locomotion (adult rat, although the cycle periods were much longer and there were variations in the chemically-induced patterns. The excitatory amino acids tended to enhance flexor muscle activity, whereas, extensor muscle activity was enhanced with dopamine suggesting a differential role of excitatory amino acid and dopaminergic mechanisms in locomotor pattern generation. These data establish that spinal motor pattern generating circuitry can be selectively activated in vitro to produce locomotor patterns resembling those generated in vivo. SUPPORTED BY NIH GRANTS HL-37941 AND NS-16333. JCS IS A P.B. FRANCIS FELLOW.
427.7 RECURRENT INHIBITION OF INTERNEURONS MEDIATING VESTIBULAR EXCITATION OF LUMBAR SPINAL RENSHAW CELLS. H.-G. Ross. Department of Physiology, University Düsseldorf, F.R.G.

Dynamic natural stimulation of the maculae modulates the discharge pattern of lumbar spinal Renshaw cells (Ross, H.-G. & Nittrock, C. Pflügers Arch. 408: R54, 1987). The present study pursues the question how ongoing activity of Renshaw cells can be integrated into this vestibulo-spinal pathway. In precollicularly decerebrate cats (no relaxation, dorsal roots intact), motor activity was simulated by electrical shocks to cut ventral roots L7 or S1, while the discharges of individual Renshaw cells (recorded with implanted micro-electrodes) were monitored during transient (falling) or cyclic (quasi sinusoidal) vertical movements of the animals (amplitude 35-40 cm, peak velocity 70-100 cm/s). Of the two phases of modulation (facilitation and inhibition of Renshaw cells) occurring during such natural macula stimulation, only the facilitation was affected by antidromic conditioning excitation of alpha motor axons, being reduced to about 50% of its control value. In contrast, the inhibitory response of the Renshaw cells was more depressed by the repetitive stimulation. This result is in line with the fact that the HD insensitivity. To test whether pre-synaptic factors play a role in the organisation of head aversion reflexes, exciting tonically contracted vastus lateralis (VM) oligosynaptically, would be even more depressed by the repetitive stimulation. In the present study, we have taken advantage of the ability of the anterograde tracer, PHA-L to label axons and their collaterals in Golgi-like fashion in order to test the vestibulospinal (VS) connections to the upper cervical spinal cord of the cat. In each experiment, PHA-L was injected into a discrete region of the vestibular nuclei. These injection sites included regions of the medial, lateral and descending vestibular nuclei. In contrast to previous studies, VS axons were not restricted to the ventromedial and ventrolateral funiculi but were also stained bilaterally in the lateral funiculi, the dorsolateral funiculi and in the dorsal columns. Boutons were also found bilaterally and were located in lamina II through lamina IX. Although the projections of VS axons stained following injections in different sites overlapped, no two injections resulted in identical projections. This suggests that there may be a topographical link between the location of the injection and the distribution of axons and boutons. Regardless, VS projections are more widespread than previously recognized (supported by MRC of Canada).


One of the most basic of head movements in the cat is reflex withdrawal. Consistent with this, a substantial discharge of neck motoneurons follows stimulation of the trigeminal nerve, the trigemino-neck reflex (TNR). The superior colliculus (SC) receives extensive connections from the trigeminal system and has long been presumed to play a role in the organisation of head aversion movements. The factor that limits the role of the SC is the observation that the TNR is sufficient for the reflex pathway to go through the SC. The TNR has now been more extensively analysed and evidence for the role of the SC pathway examined. In ketamine/chloralose anaesthetised cats the exposed superior colliculus was cooled while recording the TNR. No statistically significant effects either on latency, duration, peak amplitude or the form of the TNR were seen. Ablation of the superior colliculus too was without statistically significant effect on the TNR. The SC may play a role in controlling the excitability of the TNR. Microstimulation of the superior colliculus at current strengths of 20 μA was found to influence the amplitude and latency of the TNR. Supported by MRC of Canada.


Homosynaptic depression (HD) of soleus, from pairs of varicosi spaced 50 ms and 300 ms apart, was powerful in young and old men (n=21). It was anticipated that a weaker heteronymous Ib reflex, exciting tonically contracted vastus medialis (VM) oligosynaptically, would be even more depressed by the repetitive stimulation. However, no such VM depression occurred. Evaluation of homonymous H reflexes in VM revealed HD insensitivity. To test whether pre-synaptic inhibition differentially evoked the soleus HD, weak and strong stimuli were presented separately and together. The proportionality of HD to size of first pulse mitigated against this pre-synaptic hypothesis. The factor differentiating the two muscles is not clear. Tonic contraction is not responsible, as in soleus it would stop the facilitation of the muscles may be in after hyperpolarisation and neurotransmitter turnover states. Supported by The ALS Society (Canada) and NSERC grant # A0025.


Microinjection of cholinergic drugs into the pontine reticular formation of the cat activates a state with all of the components of natural REM sleep but the sites of origin of any natural cholinergic input have remained unknown. To determine these we used choline acetyltransferase (ChAT) immunohistochemistry combined with retrograde transport of horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) from small (10-30 nl) pressure injections in pontine FPTG in 4 cats (TMB processing, 2 days survival). ChAT immunoreactivity was detected utilizing monoclonal antibody AB8 and the PAP method, with DAB as the chromogen. Cell counts showed that 10.2% of the total population of ChAT-labeled LDT neurons were double-labeled ipsilateral to the WGA-HRP injection site in PFTG and 3.7% contralateral. Of all ChAT-immunoreactive neurons in the PPT, 5.2% were double-labeled ipsilateral and 1.3% contralateral to the WGA-HRP injection site. An anterograde study employing a single iontophoretic injection of PHA-L (visualized by the PAP method after 5-8 days survival, 10 cats) showed that PHA-L-positive fibres with bouton-like varicosities were present in both ipsilateral and contralateral PFTG for both LDT and PPT injections, with greater density for LDT.
428.1 RECEPTOR IMPULSE INTERVAL PATTERNS DEFINE EQUIVALENT OLFACTORY STIMULI. R. C. Gerlach, Anatomy & Cell Biology, Univ. of Cincinnati Med. Ctr., OH 45267.

When an appropriate stimulus is presented to the frog olfactory epithelium those cells which can be activated by that stimulus are excited. At low stimulus concentrations this results in a few evoked spikes followed by normal spontaneous activity. As concentration increases, the number of evoked spikes increases and so does their frequency, resulting in a shorter and more vigorous response. This is followed by a period of depressed excitability which increases in duration with concentration. With some stimulus chemicals at high concentrations of as few as 1 or 2 spikes are evoked before the onset of the quiet period. These phenomena are not due to adaptation in receptor-transducer processes rather, they result from axon inactivation due to ion concentration changes in the olfactory nerve which are not rapidly reversed. There are two useful consequences of these observations. 1 Stimulus follows excitation, durations of suppression periods are a measure of the extent to which the stimulus evokes activity in neighboring axons. At one extreme, the stimulus affects only one cell in a local region, suppression is brief and due only to ionic changes following activity in and around that cell. At the other extreme, the stimulus activates a large proportion of the population, suppression is long lasting and is a measure of the number of cells stimulated and their sensitivities. Such measurements may allow description of cell selectivity and of population coding of olfactory information.

Supported by NSB S8584023 and NSH 11233 and NSH 33348.


The [14C]deoxyglucose method was used to produce autoradiographs from the olfactory epithelium and forebrain of rats stimulated with clean or cage air, or known concentrations of the odorants ethyl acetate, propionic acid and acetaldehyde. The VIP (vasoactive intestinal peptide) receptor is restricted to the olfactory epithelium. (Supported by NIH.)

428.3 PROPERTIES OF THE OLFACTORY GENERATOR CURRENT. S. Firestein, F.S. Werblin and G.M. Shepherd, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Recordings of the generator current in olfactory sensory neurons were obtained with the whole cell patch clamp technique. The use of isolated cells (no mucus), and a technique which permitted measurement of the magnitude and time course of a pressure ejection stimulus solution at the ciliary membrane, have allowed us to uncover several fundamental measurements of the odor generated current.

1) Dose-Response Curves. Odorous substance concentrations required for threshold responses were in the 10-7 Molar range. Dose-response curves follow a sigmoidal shape and were very steep, saturating over less than 1 log unit of stimulus concentrations (Kd = 2 x 10^-7 M). 2) Response Latency. The mean latency from the arrival of the odorant substance to the onset of the current was 350 msec, range = 150-650 msec. The mean latency from the peak of the stimulus concentration to the peak of the current response was 650 msec. 3) Desensitization. The response to a maintained stimulus (20 sec) was transient with a decay half time of 9.4 seconds. Responses to short (100 msec) but saturating stimulus steps every 5 seconds showed only a 20% decrement after 20 consecutive stimulations. Supported by NS 07609 and RS 10174.

428.4 DEVELOPMENT OF A METHOD FOR PRIMARY RAT OLFACTORY NEURON CULTURE. S. Firestein, F.S. Werblin and G.M. Shepherd, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The olfactory neuron has been thus far refractory to primary cell culture. We describe a method which yields a nearly pure olfactory neuronal population. Turbinates from 2-3 day neonatal rats were dissociated and transferred to MEM, and finely minced. The tissue is pelleted at low speed and incubated for one hr at 37°C in MEM containing digestion enzymes. Then, the tissue is washed through a wine screen, and pelleted. Cells are resuspended in MEM, pelleted, and resuspended in MEM containing 5 x 10^-7 M Ca++ and 10 µM Ara C. Cell clumps are removed by filtering through 50 µM swivel filters. The resulting single-cell suspension is plated on laminin-coated dishes. After 5 days, 98% of cells remaining are bipolar, one process being long and unbranched while the other is short and divides into 6 to 8 branches. These cells stain for olfactory marker protein (OMP) and vimentin, but are negative for keratin, GFAP, S-100, and neurofilament. Based on these and other markers these cells appear to be neuronal with no evidence for glial, fibroblast and epithelial cellular contaminants. These cultures to our knowledge represent the first instance of olfactory neurons maintained in pure culture.

428.5 SINGLE-CHANNEL K+ CURRENTS IN THE APICAL MEMBRANE OF NECTURUS TASTE CELLS. C.S. Kinnamon. Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver, CO 80262.

Previous studies have shown that isolated mudpuppy taste receptor cells possess a voltage-dependent K+ current which is modulated by the presence of afferent input (Kinnamon and Aaronson, J. Gen. Physiol. 91:351-371, 1988) and is restricted to the apical membrane (Kinnamon et al., Biophys. J. 53:11a, 1988). In this study, single channel recordings in the inside-out configuration were used to identify the types and voltage dependence of the K+ selective channels involved in this conductance. A number of K+ currents were found in the apical membrane (classified by conductance values of approximately 27, 46, 53, 80, 90, 100, 110, 120, 132 and 180 pS). It is not clear that these represent fundamental differences in the K+ channels, or if some values represent variability of a single channel type. However, there are at least 5 different conductance channels because not all of these replicate in single patch. All channels showed a small probability of opening at the resting potential, and the smaller channels showed an increased probability of opening with depolarization. The largest channel was Ca++-dependent as well as voltage-dependent. Studies are underway to determine the effects of taste stimuli on the different K+ channels. (Supported by NIH grant NS 20382).


Total mRNA was isolated from olfactory mucous (30 µg/130 mg tissue) of Rana pipiens and from 14 d old rat pup brains (8.2 mg/brain). About 150 ng of olfactory mucosal mRNA responded to an odorant mixture but not to other ligands 2 d later. On the 3rd day, oocytes microinjected with Rana pilipiens olfactory mucosal mRNA responded to an odorant mixture but not to other ligands; oocytes microinjected with rat brain mRNA responded to 5-hydroxytryptamine but not to an odorant mixture. The controls were non-responsive to the odorant mixture. The magnitude of the small-amplitude (10-20 nA), long-duration (2-3 min) transient currents in response to odorant stimulation was enhanced by preincubating the oocytes with 1 µM 8-bromo cyclic AMP for 10 min. The data suggests that the channel associated with sensory transduction in olfactory receptor neurons were translated and inserted into the oocyte membrane. Further experiments will determine the usefulness of the Xenopus oocyte as a convenient and efficient transfection system for mRNA encoding membrane receptors, intermediate molecular entities and channel proteins associated with olfactory transduction. Experiments performed at Cold Spring Harbor Laboratory; supported by NIH-NS-16340.
CHEMICAL SENSES: PERIPHERAL MECHANISMS I

GENETIC FACTORS IN TASTE BUD DENSITY AND TASTE PREFERENCE. The SWR/J strain of mice avoids the bitter substance sucrose and this phenotype is heritable. Serial, paraffin sections were made and complete taste bud counts were compared. The SWR/J (taster) strain contained a mean of 169.4 (N=5, range 142-217) taste buds/mm², while the non-taster line contained only 59.1 (N=6, range 47-75). This significant difference may be attributable to variation in taste bud density. (Supported by NIH Grants NS 20101 and NS 15560).


Historically, most attempts to measure changes in the rat's gustatory sensitivity following selective gustatory deafferentation have involved the use of discriminative taste tests. These tests are contaminated by non-taste factors. Moreover, intake tests have questionable sensitivity in the detection of changes in gustatory sensitivity. To overcome these limitations, we studied the gustatory thresholds for sucrose and NaCl in rats with selective deafferentation of the geniculate ganglion. Deafferentation of the geniculate ganglion contains cells that are uniquely responsive to gustatory stimuli that are otherwise undetectable by active Na+ transport. The identity and distribution of these cells is under investigation. Since the enzyme Na+ ,K+ -ATPase mediates active sodium flux in a variety of other systems, we have used this distribution of the sodium transport mechanism(s) therefore have significance for transduction. The procedure detects phases, which is released from the taste bud, to a new receptor system that has significantly enhanced activity (Ernst, J. Histochim. Cytochem., 1972) to identify sites of elevated sodium activity in olfactory epithelium. The procedure detects phases, which is released from the taste bud, to a new receptor system that has significantly enhanced activity (Ernst, J. Histochim. Cytochem., 1972) to identify sites of elevated sodium activity in olfactory epithelium. The procedure detects phases, which is released from the taste bud, to a new receptor system that has significantly enhanced activity (Ernst, J. Histochim. Cytochem., 1972) to identify sites of elevated sodium activity in olfactory epithelium.


The olfactory organ of the Florida spiny lobster, Panulirus argus, consists of an array of aesthetasc sensilla on the lateral branch of the antennule. Each sensillum is innervated by several hundred chemosensory neurons. Electro-physiological studies show that among these neurons are cells that respond specifically to aromatic, basic, and/or aromatic amino acids. A putative amino acid receptor has been identified in antennular sensilla. The amino acid receptor is present in a population of chemosensory neurons that have been shown to transduce olfactory stimuli. Biochemical studies demonstrate that the receptor is a ligand-gated ion channel with high affinity for amino acids. The amino acid receptor mediates the opening of a cation-selective channel that is activated by amino acids and inhibited by ATP.

429.1 PROTEIN KINASE C ACTIVITY DURING CEREBRAL ISCHEMIA IN RAT CEREBRAL ISCHEMIA V THURSDAY PM

R.C. Crumrine*, J.C. LeMane, R.G. Kubtyk (SPOR; M.D. Lust) Deps. of Physiology/Biophysics and Neurology, Case Western Reserve University Medical School, Cleveland, OH 44106

Protein kinase C (PKC) can be increased by intracellular Ca2+, increased availability of diacylglycerol, and free arachidonic acid. All of these changes in the intracellular environment have been demonstrated in cerebral ischemia. In this study, we investigated the consequences of cerebral ischemia on activation of PKC. Cerebral cortex samples were removed at times 0, 5, 10, 15, 20, and 30 minutes of irreversible cerebral ischemia produced by KCl induced cardiac arrest. Rats were frozen in situ at the times indicated. We did not observe an increase in PKC activity in the membrane fraction after 5 minutes of ischemia. There was also a rising trend in the proportion of activator-independent activity in the membrane. PKC can be converted to an activator-independent form known as protein kinase M by proteolytic cleavage secondary to attack by calpains. We propose that this may be occurring during ischemia. We also noted a general trend of decreasing inducible total PKC activity during the ischemic time course studied. Activator-independent kinase activity did not correspondingly rise. This may reflect a change in PKC efficacy or a disappearance of the enzyme.


The NMDA receptor mediated excitotoxicity has an important role in hypoxic neuronal injury in vitro. In this process we examined the effects of NMDA antagonists and other receptor modulators on hypoxia-induced calcium accumulation in cultured neurons. Cortical isolates of fetal rat brain were exposed to a 95% N2/5% CO2 atmosphere for four hours. Prior to hypoxic exposure, maintenance medium was replaced with Hanks' balanced salt solution containing varying concentrations of test compounds. Intracellular calcium accumulation was quantitated by adding trace amounts of 45Ca to the test medium. TTX (3 μM)/MPA+ (10 μM) controls were used to estimate hypoxic Ca++ accumulation in glucol cells. The competitive NMDA antagonists 7-AP and CPP blocked 45Ca++ accumulation with IC50s of 10 and 4 μM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 μM, respectively. Each antagonist suppressed calcium accumulation to near TTX/MPA+ control levels. NMDA antagonists prevent hypoxia-induced calcium accumulation in cultured neurons.


Reports of neuroprotection conferred by calcium channel (CC) blockers in cerebral ischemia have been variable and inconsistent. In this study we assessed (S)-emopamil (E), a highly permeable agent with both CC and serotonergic receptor blocking properties. In 32 fed adult male Sprague-Dawley rats, middle cerebral artery ligation was followed by 210 min of hypoxia-ischemia. E reduced infarct volume to 31-38% of control (p<0.01) given twice a day until perfusion-fixation at 3 days. Brain infarct areas were assessed (S)-emopamil (E), a highly permeable agent with both CC and serotonergic receptor blocking properties. In 32 fed adult male Sprague-Dawley rats, middle cerebral artery ligation was followed by 210 min of hypoxia-ischemia. E reduced infarct volume to 31-38% of control (p<0.01) given twice a day until perfusion-fixation at 3 days. Brain infarct areas were assessed.


Intracerebral calcium accumulation was quantitated by adding trace amounts of 45Ca++ to the test medium. TTX (3 μM)/MPA+ (10 μM) controls were used to estimate hypoxic calcium accumulation in glucol cells. The competitive NMDA antagonists 7-AP and CPP blocked 45Ca++ accumulation with IC50s of 10 and 4 μM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 μM, respectively. Each antagonist suppressed calcium accumulation to near TTX/MPA+ control levels. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells. The competitive NMDA antagonists 7-AP and CPP blocked 45Ca++ accumulation with IC50s of 10 and 4 μM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 μM, respectively. Each antagonist suppressed calcium accumulation to near TTX/MPA+ control levels. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells.

429.5 MONOSIALOGANGLIOside GMI REDUCES APOPTIC NEURONAL DEGENERATION IN VITRO. R.D. Skaggs, A. Facal, D. Milsark and A. Leum. Fidia Research Laboratories, Abano Terme, Italy.

Glutamate neurotoxicity (GNT) may participate in the neuronal cell loss associated with neurological insults such as epilepsy, axonia and stroke. GNT has been described in cortical and hippocampal neurons in vitro, and cell death underlies the degenerative loss of the orthodromic population spikes (OPS) (normoxic) or 95% N2/5% CO2 (hypoxic) in the presence of MK-801 (10 μM). The protective effect of MK-801 against hyperoxic injury is of interest in the context of the excitotoxicity. We show selective vulnerability in normoxic-exposed cultures of granule cells from day 8 rat cerebellum. Chemical anoxia, in cells between 8 and 12 days in vitro, was produced by a pulse addition of rotenone (0.1 μM for 1 hr) in the absence of Mg++. Widespread degeneration of neuronal cell bodies and their neurite network was seen the following day. This neuronal injury was abolished by the specific NMDA receptor antagonist PCP, suggesting an action of endogenous glutamate at this receptor. We noted the time course of degenerative lesion by finding the extent of the orthodromic population spikes (OPS) (normoxic) or 95% N2/5% CO2 (hypoxic) one hour after 48 min of hypoxic injury. In 2.4 mM Ca, slices recovered 12±8% of the orthodromic population spikes (OPS). MK-801 (10 μM) increased recovery to 100%. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells. The competitive NMDA antagonists 7-AP and CPP blocked 45Ca++ accumulation with IC50s of 10 and 4 μM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 μM, respectively. Each antagonist suppressed calcium accumulation to near TTX/MPA+ control levels. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells.


MK-801, a potent noncompetitive NMDA receptor antagonist, reduces neuronal hypoxic injury. We tested the role of extracellular calcium in this process, using hippocampal slices perfused with artificial cerebrospinal fluid (ACSF) containing (mM) CaCl2, 2.4 or 0; glucose, 4; and amino acids as 95% N2/5% CO2 (normoxic) or 95% N2/5% CO2 (hypoxic). One hour after 48 min of hypoxic injury in 2.4 mM Ca, slices recovered 12±8% of the orthodromic population spikes (OPS). MK-801 (10 μM) increased recovery to 100%. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells. The competitive NMDA antagonists 7-AP and CPP blocked 45Ca++ accumulation with IC50s of 10 and 4 μM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 μM, respectively. Each antagonist suppressed calcium accumulation to near TTX/MPA+ control levels. NMDA antagonists prevent hypoxia-induced calcium accumulation in glucol cells.


In 7-day-old rats, bilateral carotid ligation and exposure to 8% O2 (8) min causes extensive necrotic and hippocampal damage. The inner layer of hippocampal granule cells (HGC) is selectively vulnerable, while CA3 pyramids are resistant. HGC vulnerability decreases with age and after 18 days they are resistant to the same treatment, while CA3 becomes vulnerable. Immunocytochemical staining with polyclonal antibodies to calbindin D28K (calcium-binding protein, CABP), show that the vulnerable inner layer HGC at 7 days and the vulnerable CA3 cells of the adult lack CABP, while the resistant outer HGC and CA3 cells at 7 days contain CABP. While this association does not prove causality, it is compatible with a role of CABP as a buffer that limits rises in intracellular free calcium and protects neurons during hypoxia-ischemia. Supported by grants NS15151 and NS12821 from NIH and by the research service of the VA.
HYPERGLYCEMIA PROTECTS CULTURED ASTROCYTES FROM HYPOXIA.

Many of the neurological effects of hyperglycemia and the associated compensatory increase in cerebral blood flow (CBF) may be related to the increased metabolism of glucose by both astrocytes and neurons. Since astrocytes are the major glutamate scavenger in the brain, we considered the possibility that hyperglycemia might protect astrocytes against hypoxia.

We studied the effects of glucose concentration on the viability of astrocytes in primary culture. Glucose was added to the growth medium at concentrations of 0.3, 0.6, 1.2, 2.4, and 4.8 mM. After 24 h of incubation, the cells were stained with fluorescein diacetate (FDA) and propidium iodide (PI) to distinguish viable from necrotic cells and normally is more acid compared to in vitro-exposed cells. The present study examined the effect of glucose on cell viability and the associated changes in intracellular pH (pHi).

We found that glucose at concentrations of 2.4 and 4.8 mM significantly protected the astrocytes against hypoxia. The percentage of viable cells, measured by FDA uptake, was significantly higher in the glucose-treated groups than in the control groups (p < 0.05).

These findings suggest that hyperglycemia affords protection against focal cerebral ischemia and results in a 38% reduction in the size of the infarct produced by MCA occlusion. This reduction in infarct size with FN stimulation may represent a rescue of the ischemic penumbra by a drop in vascular resistance and opening of collateral flow.

IMPROVING CEREBRAL BLOOD FLOW DOES NOT IMPROVE METABOLICninger in the medulla (100.9 ± 10.7 ml/100 g/min) and decreased in frontal CTX. These data document a redistribution of cerebral blood flow during focal cerebral ischemia. These data suggest that hyperglycemia and hyperosmolarity may reduce metabolic needs and prevent pathologic change. This may reduce metabolic needs and prevent pathologic change.

70 KDa STRESS/HEAT SHOCK PROTEIN INDUCTION IN GERBIL BRAIN AFTER ISCHEMIA IS BLOCKED BY THE ANTI-INFLAMMATORY, MK-801. T.S. Nowak, Jr., Laboratory of Neuropathol. and Neuroanat. Sciences, NIH/NIH, Bethesda, MD 20892

Immunocytochemical studies have localized induction of the 70 KDa stress protein (hsp70) in gerbil brain after transient ischemia. Accumulation is most striking in CA1 neurons of hippocampus at 48 h recirculation, preceding the loss of functional neurons characteristic of this model. MK-801 has been suggested to block ion channels associated with glutamate receptors of the N-methyl-D-aspartate (NMDA) receptor and thereby protect neurons from excitotoxic cell loss. The present study examined the effect of MK-801 on hsp70 induction and CA1 damage following transient cerebral ischemia in gerbil. Pretreatment with 10 mg/kg MK-801 1 h before 5 min ischemia prevented the appearance of hsp70 immunoreactivity in CA1 at 24 h. However, pretreatment at 10 mg/kg 1 h before 5 min ischemia attenuated the induction. In contrast, no protection of CA1 neurons was evident after either treatment when evaluated immunocytochemically at 1 week. These results fail to support a protective effect of MK-801 on postischemic cell damage in CA1, and clearly dissociate the induction of hsp70 in CA1 from functional neuronal injury. The results suggest further studies of the physiology resulting in such localized changes in gene expression after ischemia.
A NOVEL METHOD FOR TARGETING NEURONS IN A LIGHTLY ANESTHETIZED MICE Using FIBER OPTIC PROJECTION R.T. Walker*, A.M. Graybiel, R.W. Baughman and G.W. Arbuthnot** (SPO: H. N. C. and G. W. A.) Here we report a method for using a fluorescent Nissl stain (see Quinn and Weber, this meeting) that permits targeting of specific cell types according to the appearance of their perikarya, combined with injection of Lucifer Yellow, visible at the same wavelength. This method works best in a lightly fixed and retinal slice preparation. Slices 40-100 µm thick were taken from perfused ferret brain and maintained in the same condition. A microscope fitted with a 10x objective was used to focus on a single cell and inject Lucifer Yellow into the nucleus of the cell. Lucifer Yellow was injected into a single cell and the site of injection was then scanned in a number of cases. The method was found to be reproducible with the use of the same microscope and Lucifer Yellow was injected under direct vision into the cell of choice. After a number of cells had been filled in a slice, it was post-fixed by immersion in 4% paraformaldehyde, and cut into 100-150 µm thick sections which were then stained for cholinesterase. This method enables reconstruction of dendritic fields of striatal cells with respect to the histologically definable structures, and should have broad applicability to the study of other regions of the central nervous system.

Supported by the Faculty of Medicine, Uni. of Edinburgh, the Whitaker Health Sciences Fund and NSF BNS 870475.

SHIFTS IN CLASS DISTRIBUTION OF TH-LABELLED CELLS IN THE SUBSTANTIA NIGRA OF THE MUTANT MOUSE WEAVER. A.M. Graybiel, S. Roffler-Tarlov. (SPO: D. Chikarashie) Dep. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139. The neural systems related to motor behaviour are also afflicted with the weaver gene in mice. Recent work has confirmed that the weaver gene in mice is remarkably specific, discriminating between the nigrostriatal and mesolimbic pathways so that the nigrostriatal targets are profoundly affected and the mesolimbic targets are relatively or completely spared.

We report here measurements of dopamine release in the mesostriatal and mesolimbic pathways, using a radiofluorimetric method of dopamine-containing terminal function, in the three striatal regions in weavers and controls. Synaptosomes were prepared from caudoputamen (CP), nucleus accumbens (NAc) and olfactory tubercle (OT). These preparations were incubated with [3H]dopamine, and dopamine uptake was measured by the fluorimetric method.

The results revealed an even greater deficit in dopamine uptake capacity in weaver than in control levels, with controls being 100% of normal, whereas weavers had uptake levels of 65% of normal.

The present study examined GAD mRNA as related to the increase in stria
tal GABA synthesis in weaver mice. We report here measurements of dopamine release in the mesostriatal and mesolimbic pathways, using a radiofluorimetric method of dopamine-containing terminal function, in the three striatal regions in weavers and controls. Synaptosomes were prepared from caudoputamen (CP), nucleus accumbens (NAc) and olfactory tubercle (OT). These preparations were incubated with [3H]dopamine, and dopamine uptake was measured by the fluorimetric method.

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430.7  

The purpose of the present experiments was to determine whether fetal neural tissue are able to reverse the alterations in host striatal GABAergic function produced by 6-hydroxydopamine (6-OHDA). Rats with unilateral 6-OHDA lesions at birth were transplanted at 16 days of age with fetal substantia nigra tissue placed into cortical cavities. 8 weeks after transplantation, striata were removed and a radiochemical assay was used to determine glutamic acid decarboxylase (GAD) activity. There was no difference in the GAD activity between the two striata in non-lesioned rats, whereas there was a marked increase (>40%) in striatal GAD activity homolateral to the 6-OHDA lesions in rats without transplants. In contrast, in rats with successful transplants striatal GAD activity was similar to the non-lesioned control values. Successful transplants were those which reduced dopamine release and restored GABAergic activity as judged by the 6-OHDA lesions. Supported by grants NS 21003, BNS 86-07645 and BNS 86-16841.

430.8  
ULTRASTRUCTURAL LOCALIZATION OF MOLECULAR SUBTYPES OF NEURAL CELL ADHESION MOLECULE (NCAM) IN THE ADULT RODENT STRIATUM. M. Delpillia, F. Mitchell and M. Yamada-Price. Departments of Neurology, Massachusetts General Hospital, Boston, MA 02114 and Biochemistry, Eugene Kennedy Driver Ct., Valhalla, NY 10595.

Neural cell adhesion molecule (NCAM) belongs to a class of integral plasma membrane glycoproteins which are thought to mediate adhesion between neuronal elements. We used immunohistochemistry (avidin-biotin-peroxidase) to examine the localization of immunoreactive (I) NCAM in the adult rodent striatum. The monoclonal antibody used in this study was raised against embryonic (E15-17) mouse brain and identified E15, 140 kd molecular weight forms of NCAM. Light microscopic results in the adult male (66) and rat (120) showed that NCAM was localized to the plasma membranes of somata and dendrites of medium and large-spired neurons throughout the caudate nucleus and to the majority of neurons in the globus pallidus. Ultrastructural analysis revealed that reaction product in axon somata was present in discrete, closely spaced patches along the inner face of the plasma membrane and was also prominent in somatic protrusions which were frequently opposed to synaptic areas. Dorsal aminergic dendrites with NCAM received numerous synaptic inputs. Within caudate neuropil IHCW was also present in 1) medium spiny neurons where reaction product was localized predominantly to spines with, long thin necks and small spine heads which were postsynaptic to unlabeled axon terminals, and 2) pretentorial (dendritic) processes that issued from myelinated bundles and formed asymmetric synapses with unlaelled dendritic spines.

We speculate that the prevalence of NCAM in medium and large aminergic caudate interneurons and in putidial projecting cells may be involved in modulating the density of synaptic inputs, since all three types of neurons share the feature of being ensheathed by axons and terminals. The presence of NCAM in thin spines may reveal sites of plasticity in adult spiny neurons. Supported by grants NS16367 to M.H. and NS 26759 to M.Y.

430.9  

Using ISHH, we examined the regional distribution of SOM mRNA in the striatum of the mouse. Also, because lesions of dopaminergic nigrostriatal neurons stimulate striatal SOM neurons, we determined the effect of dopamine (DA) receptor blockade on the levels of SOM mRNA. Male Swiss Webster mice were injected 2 times, 8 hours apart, for 2 consecutive days with a dose of phenolamine-N-mustard (PNM, 4 mg/kg), which irreversibly blocks DA-D2 but not D1 receptors. The right striata were analyzed by DOPAC and DA using HPLC-EC. The DOPAC to DA ratio, an index of DA turnover, was elevated after PNM treatment, confirming effective blockade of DA-D2 receptors. The left side of the brain was processed for ISHH.

Levels of SOM mRNA were quantified using light microscopy and computer-assisted grain analysis. In controls, individual neurons of the lateral striatum had higher levels of SOM mRNA than did those of the medial striatum. FNMT treatment produced a decrease in SOM mRNA levels in the lateral, but not the medial striatum. The results indicate that there is a lateral to medial gradient in the levels of SOM mRNA in the striatum (of control animals). FNMT treatment taken together with data from the literature, these results suggest that activation of the nigrostriatal pathway by sustained DA-D2 receptor blockade produces a region-specific reduction in striatal SOM gene expression. Supported by BNS 86-07645 and BNS 86-16841.

430.10  
PERSISTENCE OF POLYSIALYLATED ("EMBRYONIC") N-CAM IN THE SUBSTANTIA NIGRA OF ADULT RATS. M.-F. Chesselet and L.I. Aaron*.


The embryonic (E) form of the Neural Cell Adhesion Molecule (NCAM) contains a higher amount of polyosialic acid residues than adult N-CAM (A-N-CAM). As a result, binding between A-N-CAM is much stronger than between the E forms of the molecule. Maturation from E to A-N-CAM is believed to be a critical developmental event, but its chronology has not yet been studied at the anatomical level. We have detected the highly polysialylated N-CAM in sections of the rat brain by indirect fluorescence immunohistochemistry, using a monoclonal antibody (from C. Reutger) raised against the capsular polysaccharides of meningococcus B. This antibody reacts with the E but not the A-N-CAM. In the substantia nigra (SN), the labeling was intense and homogeneous in sections from 1-18 day old rats, with both cell membranes and intersomata space showing immunoreactivity. At 16 days, a ventro-dorsal gradient in the level of immunostaining appeared in the SN pars reticulata. Also, immunolabelling of cell membranes became increasingly discontinuous, taking on a more punctate appearance in sections of 3 weeks old rats. Immunostaining was still present in the SN of adult rats, but was found mostly between cell bodies. Labeling in the adult SN contrasted with the lack of Immunostaining of most other brain areas at this age. Notable exceptions in the brain stem were the periaqueductal grey and the lateral subventricular of the interpeduncular nucleus. At all age examined, intense and widespread immunoreactivity to a polyclonal antibody recognizing both A and E-N-CAM was found throughout the brain. It is proposed that highly polysialylated N-CAM may play a role in neuronal plasticity in discrete areas of the adult brain, including the SN. Supported by BNS 86-07645 and the Dystonia Medical Research Foundation.

430.11  

Noradrenergic input to the rat substantia innominata (SI) was investigated in this study by immunocytochemical localization of dopamine B-hydroxylase (DBH), the synthetic enzyme for noradrenaline. Using a rabbit anti-bovine DBH antiserum (Eugene Tech International), dopamine B-hydroxylase (DBH+) elements were revealed by a standard avidin-biotin-HRP immunoreaction. DBH+ axons ramified extensively in SI and appeared to be contiguous with DBH+ terminal fields within the bed nucleus of stria terminalis and the amygdala complex. Individual DBH+ boutons in SI appeared much larger than those in the cerebral cortex, hippocampus, and thalamus. Electron microscopic analysis revealed that DBH+ boutons in SI contained many small clear synaptic vesicles (30-60 nm) and large uniformly stained vesicles (80-120 nm). DBH+ boutons formed asymmetrical synapses with mainly dendrites, but also soma and spines of SI neurons. Dendrites which were postsynaptic to DBH+ boutons also formed many symmetrical and, in some asymmetrical synapses with unlabeled axon terminals. Since previous studies have shown that cholinergic neurons formed few synapses, the present results suggest that noradrenergic influence of SI cholinergic neurons, if present, is through polysynaptic connections. Supported by grants NS21203, AG05944, and a grant from the Alzheimer’s Disease and Related Disorders Association.)

The phasic electrical activity in multunit activity (volleys) associated with the injection of gonadotropin-releasing hormone (GnRH) pulses were studied in adult female rhesus monkeys (Macaca mulatta). The GnRH release rates were monitored by recording action potentials in the median eminence (MEM) using toggle stimulator. The voltage threshold for the detection of a volley was set at 100 mV. The volleys were defined as action potentials with a duration of more than 2 ms. The GnRH release rates were calculated by measuring the area under the curve of the action potentials. The GnRH release rates were significantly higher in the morning hours compared to the afternoon hours. The GnRH release rates were also higher in the group of monkeys that were on a high-protein diet compared to the group that was on a low-protein diet. This study provides evidence for the existence of a circadian rhythm in the GnRH release rate in female rhesus monkeys.
341.1

**EFFECTS OF PRAZOSIN ON PULSATILE LUTEINIZING HORMONE (LH) RELEASE IN CASTRATE, RSH ENHANCED, EVENLY AGED MALE RATS.**

M. F. Salomonoff, C. Chu*, L. E. Peterson, C. A. Barracough and R. T. Zoeller, Dept. of Physiol., Univ. of Maryland, Sch. of Medicine, Baltimore, MD 21201 and LNC, NIH, Bethesda, MD 20892.

We quantitated single cell mRNA levels in brains of intact and castrated rats and in castrated rats bearing the 37311 A 1-receptor containing tumor. Single cell postcastration, rats were decapsulated and 12 mm sections prepared from the diagonal band of Broca through the mediad preoptic nucleus (MPN). Sections were hybridized with 35S-labeled de-ss-oligonucleotide probe. A single section in the region of the MPN was chosen from each brain and quantitated using dark field optics in a Scan System IV. The average number of cells labelled/sample was 8.9 ± 0.5 (n=21) and the average cellular labelling intensity was 175 ± 18.7 (n=21). After 7 days, both groups of rats were injected with saline and sampled at 10 min intervals for 2 h. The data indicate that testicular steroids and PRL affect steady state GnRH mRNA levels in cells residing in the region of the MPN. Supported by NIH grants HD-21351 and HD-02138.

341.11

**ESTRADIOL INHIBITS SEPTAL NEURONS PROJECTING TO MEDIAN EMINENCE.**

S. D. Donenfeld and A. F. Ferguson, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The perikarya of luteinizing hormone releasing hormone (LHRH) immunoreactive neurons that project to the median eminence are in a continuum from the septal region through to the anterior hypothalamus. Recordings have been obtained from neurons in the septal-preoptic area that could be antidromically identified as projecting to the median eminence and have been suggested that these identified neurons are LHRH neurons. In the female rat, putative LHRH-containing preoptic neurons which project to the median eminence are regulated by systemic estrogens suggesting an inhibitory role for this steroid in the control of LHRH secretion. The present study examines, in the male rat, the effect gonadal steroids on medial septal neurons projecting to the median eminence of the male eminence. In anesthetized male rats, extracellular recordings were obtained from 32 medial septal neurons antidromically identified as projecting to the median eminence. In three experiments, was followed by a period of inhibition (100 ms), which frequently persisted (83%) when the stimulus delivered to the median eminence was below threshold for antidromic activation by systemic estrogens. The present report identifies 40% of identified septal neurons tested (n=10) was decreased following intravenous injection of 17β-estradiol (0.5-2 μg). This supports an inhibitory role for 17β-estradiol in the regulation of LHRH secretion in the male rat. Supported by MRC of Canada

341.3

**IN VIVO PULSATILE LH RELEASE INTO THE ANTERIOR PITUITARY (AP) OF THE MALE RAT.**


In the intact male rat, LH release is irregular with low amplitude pulses at intervals (30 min to 3 h). Post-castration hypersecretion of LH is characterized by an increase in frequency and amplitude driven by the putative LH releasing generator. We have reported that LH pulses were detected by using push-pull cannulae (PCC) placed in the AP of freely behaving adult male rats. Six rats were perfused (PERF) as intact (day 0) and again at 2, 4 and 7 d after CAST. Similarly, 12 rats were PERF on day 0 and at 3 and 7 days after CAST. LH pulses were detected in all but 2 rats (11/12). Past CAST, LH pulses were detected in all rats (23/23). LH levels were analyzed by RIA and subjected to PERLS analysis. Peak LH levels were 0.83±0.08 (PERF) and 0.64±0.08 (CAST). The data indicate that the inhibitory effect of CAST on LH release is not totally blocked by CAST. The mechanism of the inhibitory effect of CAST on LH release is not clear with CAST. The mechanism of the inhibitory effect of CAST on LH release is not clear with CAST. The mechanism of the inhibitory effect of CAST on LH release is not clear with CAST.

341.4

**DO GONADOTROPIN RELEASING HORMONE (GnRH) OR DOXAMINERGIC NEURONS IN THE SHEEP CONTAIN ESTRADIOL RECEPTORS?**


We have previously determined that sheep GnRH neurons, or tyrosine hydroxylase-(TH)-positive neurons which may be afferent to them, contain ER. Adult male rats were injected with 0.2 or 0.4 mg/kg of prazosin or vehicle. After 4 h, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses in the remaining animals, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses continued with attenuated amplitude and normal frequency.
342.1 IMMUNOPURIFICATION OF BUTYRYLCHOLINESTERASE FROM CHICKEN SERUM: MOLECULAR PROPERTIES AND COMPARISON WITH THE MUSCLE ENZYME. K.W.K. Tan, M.R. Randall and E.A. Barnard. (SPON: M. Smith) MRC Molecular Neurobiology Unit, MRC Clinical Research Centre, 20, U.K. Chicken serum butyrylcholinesterase (BuChE) was purified sequentially on an anti-BuChE immunofinity column and a tritylmethylcarbinol column. The purified enzyme has a specific activity of 125 U/mg of protein on acetylcholine as substrate, representing over 13,000-fold purification. The purified serum BuChE contains one type of catalytic subunit, apparent Mr. 76,000, which can be labeled with [3H]dipropylfluorophosphate (DFP) through a trypsin digestion with N-glycanase digestion of both enzymes leads to an identical size in SDS-PAGE. Over 95% of the purified enzyme was recovered, as shown by each other in SDS-PAGE. The apparent Mr of 150,000 in the non-reduced state.

342.2 PHYSOSTIGMINE PROTECTS RAT HIPPOCAMPAL SLICE FROM INDUCED EPILEPTIFORM BURSTING. M. Abou-Dona, L.S. Jones, D. Lapadula and D.V. Lewis. Deps. of Pharmacoology and Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27710. We have shown previously that DFP produces rapid, irreversible diisopropylphosphate-induced membrane depolarization of hippocampal slices, while having little effect on the response of CA1 (Soc. Neuro. Abs. 13:1354). Results with atropine suggested that the effects were partially mediated through the muscarinic cholinergic receptor; further evidence that DFP effects were cholinergic was needed. Using 625 pM slices from adult rat brain, we found that DFP cut the epileptiform burst. In the usual manner, we have examined the ability of physostigmine (10 uM), a reversible AChe blocker, to protect the slice from the effects of DFP. Results indicate that, though the effects become somewhat hyperexcitable in the presence of physostigmine, the effect is reversible with washing. Further, when DFP is added to slices that are already bathed in physostigmine, the slices do not burst as they do in DFP alone, and the hyperexcitability is still reversible, which is not seen with DFP alone. These results suggest that cholinergic system activation as the primary mechanism for DFP-induced bursting in CA1. (Supported in part by NIES Grant No. ES0217).

342.3 NEUROCHEMICAL DIFFERENCES IN THE CAT VISUAL SYSTEM AFTER ANTICHOLINESTERASE AGENTS. A.W. Kirby, A.T. Townsend, R.G. Stafford and T.H. Harding U.S. Army Aeromedical Research Lab., P.O. Box 577, Ft. Rucker, AL 36362-5292. We reported previously that the anticholinesterase agents diisopropylfluorophosphate (DFP) (Brain Res. 325: 357), and physostigmine (Science 221:1076) have similar effects on the cat visual evoked response (VER). Recent retinal release studies suggest that DFP has a direct effect on neuronal membranes (Soc. Neurosci. Abstr. 13:1057). We report here retinal and cortical neurochemical differences following 10 min. of DFP exposure in the two eyes.

Retina and visual cortex were removed from anesthetized and paralyzed adult cats before and after physostigmine, analyzed with thin-layer chromatography, and compared to earlier results with DFP.

Previous results with DFP suggest that cortical dopamine (DA) turnover (avg. 137±7.5 x 10^-9 M) as well as retinal DA (avg. 8.28% were linked to VER changes. Following physostigmine, there is no consistent change in DA turnover, and GABA increases in cortex (avg. 96%). Retinal DA decreases (avg. 15%). Other differences are seen in cortical glycine, aspartate (asp), epinephrine, and norepinephrine, as well as retinal asp, glutamate, and GABA.

Since the VER and cholinesterase changes are similar, the neurochemical differences following the two agents are difficult to explain. Since both have cholinergic effects, membrane effects of DFP might account for the differences.

342.4 COMPARISON OF IN VITRO ANTICHOLINESTERASE PROPERTIES OF PHYSOSTIGMINE AND PHYSOSTIGMINE DERIVATIVES. L.J. Atack, B.S. Ross and R.J. Elcombe (SPON: J. Noronha). Labs of Neuroscience, NIA and Analytical Chemistry, NIDDK, NIH, Bethesda, MD 20892. Physostigmine (vesine), which is a carbamate ester alkaloid and a potent inhibitor of acetylcholinesterase (AChe) and butyrylcholinesterase (BuChE), has recently been used in the experimental treatment of Alzheimer's disease with large disappointing results. However, since physostigmine has a plasma half-life of less than 30 min., physostigmine derivatives with longer half-lifes may be more clinically useful than physostigmine itself. As a first step in the identification of such compounds, we synthesized two series of physostigmine analogues which were either carbamate- or N(1)-substituted derivatives. The in vitro potencies (IC50's) of these compounds against human brain and erythrocyte ACHE and human brain and plasma BuChE were compared to that of physostigmine. For each compound, the IC50 against human brain and erythrocyte ACHE was about 5-10-fold less than for human brain BuChE, suggesting that with respect to inhibitor susceptibility, human ACHE is similar throughout the body whereas BuChE is not. With respect to human brain ACHE, 6 compounds (octyl-, butyl- benzyl- and N-pheynl-carbamoyl aseroline and N(1)-nor- and N(1)-allyl-physostigmine) had IC50's (ranging from 15±2 to 37±4 x 10^-9 M) similar to the IC50 of physostigmine (31±8 x 10^-9 M). Of these compounds, only N-phenyl carbamoyl aseroline was a relatively selective inhibitor of ACHE rather than BuChE (IC50's in human brain ACHE and BuChE =36±3 and 2500±1100 x 10^-9 M, respectively). Eseroline, which is presumably the major metabolite of physostigmine, was a very poor anticholinesterase.

342.5 TETRAHYDROAMINOACRIDINE (THA) EFFECTS ON BEHAVIOR IN RODENTS. D. S. Chapin and J. A. Nielsen (SPON: L. K. Torgersen) 'Pfizer' Central Research, Groton, CT. Senile dementia of the Alzheimer type (SDAT) is a neurodegenerative brain disorder characterized by severe memory impairment. A decrease in neurochemical markers associated with cholinergic neurons has been found in SDAT patients. This suggests that cholinergic neurotransmission may be compromised in SDAT and that physostigmine to enhance cholinergic function may be of benefit. ThA, a potent cholinesterase inhibitor, produced symptomatic improvement in a limited trial with SDAT patients (Ma et al. [1984] Proc. Natl. Acad. Sci. 81, 3759). It was found that ThA was relatively selective for standard animal testing procedures, we have assessed the effect of ThA in rodent models and estimated the toxic effects by the lack of autonomic and behavioral side effects.

ThA reversed scopolamine-induced amnesia in a T-maze procedure after i.p. (3.2 mg/kg) and p.o. (392 mg/kg) treatment in rats. The drug also improved the performance of mice in a passive avoidance model of memory function after 3.2 mg/kg, i.p. ThA produced hypoactivity, which was reversed with physostigmine and dipropylfluorophosphate (DFP) (3.2 mg/kg, i.p.) at 17.8 mg/kg i.p. All of these side effects were prevented by scopolamine, a general muscarinic receptor antagonist which also blocks muscarinic antagonist glycopyrrolate inhibition only salivation.

These results suggest that ThA is active in rodent models of memory and involves the side effects of ThA observed in rodents probably have both peripheral and central nervous system components.
342.7 EFFECT OF THA ON ACH RELEASE IN ANIMALS WITH CHOLINERGIC LESIONS. E.E. Potter* and S. Nittou, Dept. of Pharmacology, Albert Einstein College of Medicine, Montefiore Hospital, 111 E. 110th St., Bronx, N.Y. 10467

It has been reported that tetrahydroaminoacridine (THA), which decreases acetylcholine (ACH) release from normal brain, may enhance ACH release from brain slices of patients with Parkinson's disease, whose substantia nigra was lesioned. On the other hand, cholinergic neurons in rat cortex by bilateral injection of 100 mmoles quinolinic acid into the substantia nigra, or in lesioned striatal cholinergic neurons by intraventricular administration of 2 mmoles of eel cholinergic mustard aziridinium (AF64A). The effects of THA (5 x 10^-3 M), physostigmine (10^-4 M), and LF-14, a 3,4-diaminopyridine derivative, caused a large increase in ACH release in hippocampal and cortical slices taken from control and lesioned rats. The slices were stimulated 3 times. Drugs were added between S2 and S3, and results are expressed as the S2/S3 ratio in hippocampus (Control= 0.65±0.07) and cortex (Control= 0.90±0.06; THA= 0.37 ±0.09, physostigmine= 0.46±0.02). LF-14, a 3,4-diaminopyridine derivative, caused a large increase in ACH release (S2/S3 ratio in hippocampus- 4.51±1.35; cortex= 1.24±0.18). There was no significant difference in any of these values in slices from rats lesioned with AF64A or with quinolinic acid.

342.9 MEASUREMENT OF ACETYLCHOLINE BY HPLC WITH ELECTROCHEMICAL DETECTION AND EFFECTS OF DOPAMINERGIC AND CHOLINERGIC AGENTS ON ACETYLCHOLINE LEVELS. F.P. Bymaster* and D.T. Wong (Spon: L. Truex). Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46225.

Acetylcholine (ACH) and choline (CH) are difficult to measure in tissues. Potter et al. (1983) and Bymaster et al. (1985) have reported methods for measuring ACH and CH using HPLC with electrochemical detection. CH and ACH in tissue extracts were separated, and choline oxidase and acetylcholine oxidase were added to the eluate. The hydrogen peroxide formed was quantitatively detected. A new method uses enzymic immobilization on a column, resulting in a highly sensitive ACH assay. The ACH was treated with the dopamine agonists pergolide (0.3 ng/kg), quinpirole (1 ng/kg) and LY163502 (10 ng/kg) a cholinergic agonist, raised ACH levels in vivo to 228%, respectively. Dopamine antagonists haloperidol (0.3 mg/kg) and fluphenazine (1 ng/kg) lowered ACH levels to 62 and 64%, respectively. Metoclopramide (0.25 mg/kg), a cholinergic agonist, lowered ACH levels in vivo to 18%. Physostigmine (0.5 mg/kg), an acetylcholinesterase inhibitor, raised ACH levels to 202%. Cholinergic antagonists eslicarplug (0.6 mg/kg) and atropine (10 mg/kg) lowered ACH levels to 56 and 23%, respectively. Thus it appears that cholinergic and dopaminergic agonists decrease ACH turnover, whereas the antagonists increase turnover.

342.11 EFFECTS OF LANTHANUM, HIGH POTASSIUM AND o-LATROTOXIN ON INTRACELLULAR CALCIUM AND ACETYLCHOLINE IN SYMPTOMATOUS H. W. Schoen*1, L. Rosenenthal*2 and B. Coller*2. Depts. of Pharmacology, Université de Montréal1 and McGill University2, Montreal, Canada; Istituto Scientifico San Raffaele, Milano, ITALY.

At the neuromuscular junction, La3+ has a dual effect on acetylcholine (ACH) release: it augments stimulated release is blocked. To determine effects of La3+ in the CNS, rat cortex symposomal preparations were exposed to 100 mM La3+ (30 min) or no La3+. The increase in intrasynaptosomal Ca2+ elicited by high K+ or a-LTX was significant. La3+ did not block the increase in ACH release, measured in medium following a 10 min incubation in high K+ or a-LTX. The increase in intrasynaptosomal Ca2+ elicited by high K+ or a-LTX was significant. The decrease in ACH release was blocked by La3+. The reduction in synaptosomal ACh content affected by La3+ and intrasynaptosomal Ca2+ elicited by high K+ or a-LTX was significant. La3+ had marked effects on either basal ACh release or contents. These results indicate that while in non-stimulated cortex or brain ACh release is not affected by ACh release or contents, La3+ does inhibit changes in ACh and intrasynaptosomal Ca2+ elicited by high K+ or a-LTX. Effects of La3+ on ACh release or contents were significantly reduced with either of these two agents.

It has been reported that diflunisal (5-(2,4-difluoro- salicylic acid) inhibits mitochondrial oxidative phosphorylation in Torpedo electric organ. Thus, diflunisal, 40 micromolar, and therefore the release of intramitochondrial calcium and also that diflunisal behaves as an ionophore molecule (Chadwick, E., et al., C. G., Cell, H.A. Life Sci. 37(16):1491-1498, 1985). Segments of intestine from 125 g male Wistar rat were prepared for isometric recording. It is shown that dilution, from Merck Sharp and Dohme de Mexico, causes a contraction followed by a pronounced relaxation in intestinal muscle. In addition this report presents evidence that diflunisal modifies acetylcholine induced contraction. When diflunisal is applied during an acetylcholine contraction their effects are magnified. Diflunisal actions might be promoted by the release of an accumulated calcium of the acetylchon- dria. These results also suggest an ionophoretic-like action at the muscle membrane, while a presynaptic action is not discarded, or an interaction with the acetylcholine receptor.

QUANTITATIVE CHOLINERGIC EVALUATION OF SPINAL CORD IN TOMATO CULTURES. With Friends: Chiang, Geula, Shinji Ishimoto, and John R. Delfs. Department of Neurology, Harvard Medical School, Arnold Pain Center, Middlesex Deaconess Hospital, and Dana Institute, Beth Israel Hospital, Boston, MA 02215

Anatomical and histological integrity of transverse sections of spinal cord in organotypic roller tube culture has been reported previously (Delfs and Sacoff, Soc. Neurosci. Abst. 12:528, 1986). The purpose of this work was to determine whether cholinergic neuron morphology and biochemistry is quantitated in those cultures. Cultures were studied after three weeks in vitro. For analysis of neuron size, intact cultures were stained for acetylcholinesterase (ACE) activity and the position of ACE-positive ventral horn neurons measured. Areas ranged up to 1635 with an average of 245 +/- 104 square microns which is comparable to values in vivo. Biochemically, pooled cultures showed acetylcholinesterase activity of 0.47 +/- 0.19 and AChE activity of 2.2 +/- 0.1 pmol/min/g protein (n=7), values comparable to those reported for neonatal rat spinal cord. These cultures can be used to study factors affecting ventral horn cholinergic neurons in vitro.


In order to identify genes affecting cholinergic metabolism and regulation, we have been isolating mutations that confer resistance to cholinesterase inhibitors such as the pesticide Aldicarb. Previous studies have shown that at least 12 different genes can mutate to give resistance. One of these genes is the cha-1-unc-17 complex, which has shown to be the structural gene for choline acetyltransferase (the acetylcholine biosynthetic enzyme). Other resistance loci include unc-13, unc-18, unc-33, unc-35, unc-63, unc-44, unc-65, and ion-5. We believe that many of these genes may affect acetylcholine metabolism, release, or function. We have isolated more than 400 independent spontaneous and transposon inserted resistant mutants from several starting strains known to contain active transposons; we expect that many of these mutants result from transposon insertions. Many of these mutants are severely uncoordinated or paralyzed, while others have only slight behavioral defects. Mapping experiments indicate that this set includes alleles of most or all of the known resistance loci (including at least 7 new alleles of cha-1-unc-17), plus at least one previously uncharacterized resistance locus on Linkage Group IV.

ENDOGENOUS ACETYLCHOLINE (ACh) RELEASE IN VIVO: MEASUREMENT BY INTERCELLULAR MICRODIALYSIS AND GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY (GC-MS). M.R. Marien and J.W. Richard*. Douglas Hospital Research Center, Verdun, Quebec, Canada.

Male SD rats (275-325 g) were anesthetized with urethane (1.2 g/kg) and implanted with a microdialysis probe (BAS-Carnegie Medicine) terminating in the striatum. Under anesthesia, probes were perfused with Mg-free Ringer’s solution for 8 hours, during which time drugs were administered either i.p. or via the probe. Perfusate samples (20 min, 48 samples) were mixed with deuterated ACh (deuterated ACh) and processed for GC-MS analysis of ACh according to Wood & Pelouquin (Neuropharm. 21: 349, 1982), with modifications. All data is expressed as % of the baseline.

The purpose of this work was to determine the impact of cholinergic stimulation and inhibition on a nset of important for antihelmintic action. ACh is the major excitatory transmitter at neuromuscular junctions in nematodes, and two important commercial antihelmintic laevomycetin and pyrantel. Haemonchus contortus (H.c) were measured using a modified section electrode-balance beam system. For comparative purposes, analogous studies were conducted using a mouse strain of C57Bl/6. Results were consistent with previous reports of the effects of ACh on nematode motility, and demonstrated the sensitivity of adult female Brugia pahangi (B.p.), an accessible filament nematode that is cultured in gerbil. Results of these studies support the following conclusions: (1) nicotinic agonists stimulate a pastic contraction and sustained paralysis in H.c., while B.p. stimulate a nonpastic response (contraction, followed by flaccid paralysis); (2) neither parasite is influenced by muscarinic agonists or antagonists; (3) muscarinic antagonists exert no independent effect on parasite motility; (4) they influence responses to ACh agonists; (4) B.p. desensitize to nicotinic stimulation; (5) cross-desensitization occurs among nicotine, levamisole and pyrantel. These results suggest that potentially important differences exist between nematode and mammalian nACH receptors.
432.20

**EPIDERMAL GROWTH FACTOR (EGF) BINDING SITES IN ADULT RAT BRAIN AND PITUITARY GLAND. AN IN VITRO AUTORADIOGRAPHIC STUDY.**


Recent studies have indicated that EGF may act as neurotrophic and/or neuromodulator substance in mammalian brain. Using an *in vitro* receptor autoradiographic method, we have studied the distribution of EGF binding sites in the rat brain and pituitary gland. Tissue sections were incubated with 125I-EGF (200 pM) in the absence or presence of an excess of unlabelled EGF (200 nM) and juxtaposed against tritium-sensitive film. Autoradiographic data clearly demonstrate the discrete distribution of EGF sites in rat brain. 125I-EGF binding sites are observed in several brain areas, such as cerebral cortex, striatum, hippocampal formation, certain hypothalamic nuclei, medial geniculate nucleus, ventral tegmental area, substantia nigra pars compacta and cerebellum. In the pituitary gland, EGF receptor sites are restricted to the pars distalis. Thus, EGF binding sites are present in rat brain and pituitary gland suggesting the possible involvement of this factor in the maintenance of normal CNS functions (see Quirion et al., this meeting).

(Supported by MRC, Canada and FRSQ, Quebec.)

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433.3

**ALTERATIONS IN TYROSINE (TYR) AND TRYPTOPHAN (TRYP) CONCENTRATIONS IN RAT BRAIN AND PLASMA BY CLENBUTEROL (CLEN).**


Recent studies in our laboratory showed that the β2-adrenoceptor agonist salbutamol decreases plasma TYR and raises brain TRYP concentrations (Life Sci. 42: 853, 1988). We have now examined the effects of CLEN, a β2-agonist which more readily penetrates the blood–brain barrier. Rats were injected i.p. with either 5 mg/kg CLEN or saline and decapitated 90 min later. In some experiments, the rats were pretreated with 15 mg/kg propranolol (PROP) or saline 20 min before CLEN or saline. TYR and TRYP were assayed by HPLC with electrochemical detection.

CLEN lowered both TYR and TRYP in plasma and raised them in brain. Levels of these amino acids were either unchanged or decreased in heart, lungs, spleen and liver. These changes were only partially if at all reversed by PROP. A dose-response study revealed that the reductions in plasma TYR and the elevations in brain TRYP were equal over the range of 0.5-5 mg/kg CLEN, but the effects of the lowest dose were completely antagonized by PROP. The effects of CLEN were not blocked by the serotonin (5-HT) antagonist, methysergide, ruling out an involvement of 5-HT receptors. Preliminary results suggest that the elevations in brain TYR and TRYP levels are due to stimulation of β2-receptors, since the effects are blocked by the β2-antagonist ICI 118,551 but not by the β1-antagonist ICI 118,551. Supported by grant MH28340.
OCTOPAMINES V
THURSDAY PM

433.3 THE EFFECT OF MOMETRIAL STIMULATION ON THE ACCUMULATION OF 8-PHENYLETHYLAMINE (PE) IN THE RAT STRIATUM. M. Linch, T. A. Peterson (SPON: A. A. Boulton). Neuropsychiatric Res. Unit, Univ. of Saskatchewan, Saskatoon, Sask., Canada.

PE is a neurophysiologically active compound that occurs in small concentrations in the brain, which is rapidly metabolized by monoamine oxidase (MAO). Earlier experiments have shown that unilateral electrolytic or 6-hydroxydopamine lesion of the rat substantia nigra (SN) produced decreases in PE levels in the ipsilateral striatum; but no changes were observed after mephénytoin lesion. The objective of these experiments is to determine the extent of PE accumulation following stimulation of the SN. The effects of stimulation were determined on 12 hr, 24 hr, and 48 hr after bilateral electrolytic lesions of the SN. The determination of PE was performed by a mass spectrometrical technique. The results show that MAO inhibitor-treated rats, SN stimulation increases PE utilization and removal from the brain, or the increase in rate of DA turnover decreases the availability of phenylephrine for decomposition and synthesis of PE. Supported by Saskatchewan Health and a Saskatchewan Health Research Board Fellowship (T.A.P.).

433.5 ORIGINS OF NOREPINEPHRINE IN RAT CEREBROSPINAL FLUID. P. Mamalaki*, L. S. Brady, D. Goldstein*, and M. Herkenham (SPON: M. A. Simon Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, and Hypertension-Endocrine Branch, NHLBI, Bethesda, MD 20892.

The cerebrospinal fluid (CSF) contains informational substances whose levels fluctuate with alterations in CNS activity. Sources of catecholaminergic compounds (CGF) are poorly understood. The locus coeruleus is the main source of noradrenergic fibers in brain, and the superior cervical ganglion provides dense noradrenergic innervation of the choroid plexus. CSF was drawn from the cisterna magna in rats 4 days before and after bilateral superior cervical ganglionectomy. Bilateral electrolytic destruction of the locus coeruleus and again 4-7 days after the operations. Samples (100-200 μl) of CSF were assayed for catecholamines by liquid chromatography with electrochemical detection after batch alumina extraction. Ganglionectomy (N=5) did not significantly affect CSF levels of norepinephrine, norepinephrine (NE), and epinephrine (EPI) concentrations of NE, norepinephrine (EPI), and dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in hypothalamus, except increased NE levels after bilateral electrolytic destruction of the locus coeruleus; however, total bilateral destruction decreased CSF norepinephrine by only 50%. The data indicate that the locus coeruleus is a major— but not exclusive—source of CSF noradrenaline.

433.7 EFFECT OF MORPHINE ON EPINEPHERINE CONCENTRATION IN RAT BRAIN. M. Och*, L. N. McLeod* and M. Linch. LCS, NIAAA and LCS, NIMH, Bethesda, MD 20892.

Acute and chronic morphine (MO) administration affects the function of catecholaminergic (CA) systems within the central nervous system (CNS). Most investigators have studied effects of MO on the noradrenergic and dopaminergic systems, while only a few reports concern effects of MO on CNS epinephrine (EPI) concentrations. MO produced quantified acute and chronic effects of MO on concentrations of EPI, norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in hypothalamus, except increased NE levels after bilateral electrolytic destruction of the locus coeruleus; however, total bilateral destruction decreased CSF norepinephrine by only 50%. The data indicate that the locus coeruleus is a major— but not exclusive—source of CSF norepinephrine.


Levodopa (LD) treatment schedules have been implicated in the pathogenesis of motor response fluctuations in Parkinson’s disease. We compared continuous and intermittent LD replacement regimens in rats with unilateral 6-hydroxydopamine lesions of the ascending dopaminergic pathways; rats were divided into 15 and 30 day treatment groups. In each, 3 subgroups of 7-8 rats were treated with either: a) continuous LD (2 mg/kg s.c.); b) intermittent LD treatment, contralateral rotations induced by apomorphine (0.05 or 0.5 mg/kg s.c.); c) continuous saline (0.5 mg/kg s.c.). Animals were then sacrificed, and the striata processed for glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH) enzyme activities.

433.4 THE RELATIONSHIP BETWEEN MANIC DISORDER OF CSF AMINE METABOLITES TO CLINICAL CHARACTERISTICS AFTER LITHIUM TREATMENT. C.L. Beaudon, E. Secher, S. Contreras, MA Janssen, PW Mass. Departments of Psychiatry, The University of Texas Health Science Center, and Aude L. Murphy Memorial Veterans’ Hospital, San Antonio, TX 78284.

Despite the clinical importance of manic disorder, and the general efficacy of lithium in its treatment, relatively little information is available about biological characteristics of manic disorder on the basis of clinical parameters. We studied the CSF amine metabolites 5-HIAA and HVA in manic and bipolar depressed patients both at baseline and following four weeks treatment with lithium. Changes were examined by discriminant analysis using the Family Dysfunctional Index and Clinical Symptom Assessment. In addition to data from 18 manic patients and 30 unipolar depressed patients, baseline data were obtained from 9 healthy control subjects. In unipolar depression. Amine metabolite analyses were by HPLC with electrochemical detection. At baseline, CSF HMF, but not 5-HIAA or HVA, was higher in manic patients than in healthy controls (12.0±9.4 vs. 6.6±2.36 pmol/ml, 2.5±S.D.). After treatment, 5-HIAA was higher in manic patients than in bipolar depressed patients, and the increase in 5HIAA was greater in manic patients than in healthy controls. In manic patients at baseline there was no significant correlation between 5-HIAA and 5-HIAA. After four weeks treatment the correlations showed a correlation of 0.77, p<0.01. Whereas the correlation between change in HMF and 5-HIAA was not significant for all manic patients (p=0.30), when only clinically recovered cases were analyzed, the correlation was significant (p<0.02). Data will also be presented with respect to the change in amine metabolites and the association of change in animal metabolites with change in symptomatology.

433.6 A COMPARATIVE STUDY OF THE LEVELS OF MONOMINES IN SELECTED AREAS OF THE RAT CEREBRAL CORTEX N. Kabani*, R. W. Dykes, T. A. Reader, McGill University, Universite de Montreal, Montreal, PQ.

HPLC was used to measure the levels of monoamines (MA) in the hindlimb region of the somatosensory cortex (SS), primary visual cortex (VIS) and the anterior cingulate cortex (CING) of adult male Sprague-Dawley rats.

Large, correlated changes were observed in different months of the year. A significant positive correlation (r=0.09) was seen between changes in 5-HT and DA in the SS whereas in the CING a significant positive correlation was seen for all 3 primary MAS. In the SS there was no negative correlation between NE and 5-HT (r=0.09). A highly significant positive correlation was observed in the MA levels of the CING when compared to SS and VIS for all compounds except 5-HT and NE. Significant differences between SS and VIS were seen for NE, DA, DOPAC and HVA.

Differences between the sensory corticies and CING is consistent with the functionally distinct role of the former and a difference in the levels of MAS within SS and VIS, is suggestive of a functionally different role for MAS in these two sensory corticies. (Supported by MRC of Canada)
433.11 ENZYME KINETICS OF Dopamine INHIBITION OF ARYLSULFATASE-C. E.J. Martin* and T.J. Shickley. Dept. Pharm/Tox, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104

Arylsulfatase-C (EC 3.1.6.1) occur in nature in three distinct forms: A, B and C (ARS-C), all of which are found in brain. Little is known about the role of these enzymes in the CNS.

It has been previously reported that dopamine (DA) produces inhibition of ARS-C (Emsley and Shickley, Abstr. Soc. Neurosci. 13(2), p.1474, 1987). We have investigated the kinetics of this inhibition by DA on ARS-C.

Partially purified ARS-C (Sigma, S-1629) activity was assayed spectrophotometrically using p-nitrophenyl sulfate (p-NPS) as substrate by measuring enzymatically liberated p-nitrophenol (p-NP). Inhibition was analyzed by varying DA concentration in the presence of fixed concentration of p-NPS.

Kinetic analysis of inhibition (Dixon Plot) revealed an apparent Kᵢ for DA of approximately 600 μM, which is well below the DA concentration in limbic forebrain tissue (Anden et al. Acta Physiol. Scand. 67, p.306-312, 1966). This inhibition was found to fit a model for simple competitive inhibition. (Supported by USPHS Grant NS-26040 to T.J.S.)


Adult rats are depend upon dopamine (DA) for normal behavior: destruction of nigrostriatal bundle (NSB) results in severe behavioral deficits and, if recovery occurs, deficits can be blocked with DA agonists and antagonists. In contrast, NSB lesions produced in neonates cause no such deficits and DA antagonists have little behavioral effect. We have examined the role of residual DA in striatal function in rats lesioned during development with 6-hydroxydopamine (6-HDA). At adulthood, striatal slices were preincubated with [3H]Choline, superfused with Krebs bicarbonate buffer, and exposed to electrical field stimulation (10 Hz, 1 min). The ability of sulpiride to stimulate tritium overflow was used as an index of DA inhibition of ACh release. DA in superfusates was quantified with HPLC. Although DA overflow was reduced by 6-HDA, fractional DA overflow was increased above control levels when slices were prepared from rats lesioned at 20 days of age or as adults; no such effect was seen when lesions were made at 3 or 15 days of age. Moreover, endogenous DA appeared to inhibit ACh overflow only in slices from rats lesioned at 20 days of age or greater. These results parallel our behavioral observations and suggest that if NSB injury is sustained at a young age, normal function may develop without the need for a dopaminergic influence. (Supported by NS19608)


Unilateral injection of 6-OHDA (8 μg) into substantia nigra produces lesions in rat-mantle (ME) content in the ipsilateral striatum. Increase in ME immunoreactivity occurred only in striata with greater than 90% dopamine (DA) depletion. We have observed that the increased efficacy of DA transmission may contribute to the less extensive denervation, in view of the greater than 70% DA depletion, in DA supersensitive neurons to apomorphine (1 mg/kg, i.p.) was observed. Presynaptic compensation was examined by measuring extracellular concentration of DA in 6-OHDA lesioned striata and remaining striatal tissue. Concentration of DA in remaining striata was unchanged in striata with 60-90% DA depletions, while striatal tissue was accompanied by 60% reduction of DA release. Increased DA release was also observed in striatum contralateral to 6-OHDA lesion and was accompanied by increased DA and HVA tissue content.

In conclusion, our results indicate that DA transmission in striatum is sustained following depletion of up to 90% of striatal DA. Such synaptic homeostasis may involve increased DA release, followed by increased DA, increased responsiveness of striatal neurons to DA, and increased DA release, and receptor-mediated neurotransmitter activity can lead to alterations in neuronal gene expression.

To determine the increase in DA-striatal mRNA expression, the enzyme encoded by the mRNA was measured using a combination of Northern hybridization and enzymatic assay. The results imply that altered transmitter-mediated neurotransmitter activity can lead to alterations in neuronal gene expression.

We have previously shown that dopamine (DA) inhibits arylsulfatase-C (ARS-C) (Neurosci. Abstr. 13(2), p.1474, 1987). We have also shown this inhibition to be competitive (Neurosci. Abstr. 14, 1988). In the present study we examined the ability of the DA agonist apomorphine (APO) SK&F 82526 and LY-171555 to produce inhibition of ARS-C.

Partially purified ARS-C (Sigma, S-1629) was incubated in the presence of DA, APO, SK&F 82526 and LY-171555 using a modification of the spectrophotometric technique of Fowler and Rammler (Biochem. 3, p.30, 1964).

The DA agonist SK&F 82526 and the D2 agonist LY-171555 had no ability to block the desulfation of p-nitrophenyl sulfate at concentrations up to 1E-06M. The partial agonist APO produced inhibition similar to that of DA. These results suggest a structure activity relationship for the inhibition of ARS-C by DA and APO which is independent of the receptor-specific sites of the DA agonists SK&F 82526 and LY-171555. (Supported by USPHS Grant NS-26040 to T.J.S.)
434.2 COMBINED, BUT NOT SEPARATE, INJECTION OF D1(SKF 38393) AND D2 (LY 171555) Dopamine Agonists INTO THE NASAL MUCOSA INCREASES LOCOMOTOR ACTIVITY T.J. Walsh, D.F. Emerich and L.A. Taylor. - Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

It is well established that the nasal mucocumbens (NA) and its dopaminergic innervation from the ventral tegmental area (A10) are involved in the modulation of goal-directed motor behavior. The systemic and intracerebroventricular administration of indirect and direct acting DA agonists such as amphetamine and apomorphine increase motor activity in a DA-dependent manner. Pharmacological and neurochemical evidence indicates that there is a duality of DA receptors which are designated D1 and D2 (Kebabian, J.W. Nature, 277, 93, 1979). The functional properties of these receptors in the control of motor behavior, however, have not been well characterized.

The present study demonstrated that bilateral injection of either D1 (10 or 20 µg SKF 38393) or D2 (0.5, 1.0, or 2.0 µg LY 171555) into the NA of male Sprague Dawley rats produced no significant increases in motor activity compared to saline injected controls. However, combined administration of the D1 and D2 agonists in a "cocktail" did significantly increase motor activity in a dose-related fashion. Combined administration of 15 µg of SKF 38393 and 1.25 µg LY 171555, or 10 µg of SKF 38393 and 0.5 µg LY 171555, increased motor activity 119% - 296% over control values for up to 90 minutes following injection.

These results are consistent with recent electrophysiological studies that have shown that concurrent D1 and D2 receptor stimulation in the NA may be necessary to initiate and direct locomotor behavior. (Clark D. et al., Synapse 1, 347, 1987)

These results indicate a coordinated involvement of DA receptors subtypes in the modulation of behavior.

Supported by NIH grant # ES04626 to T.W.  

434.4 CHARACTERIZATION OF RABBIT POLYCLONAL ANTI-IDiotYPIC ANTI-BODY (AB2) AGAINST CONJUGATED DOPAMINE ANTI-DOPAMINE ANTIBODY (Ab) Y. Mirochet, B. Guerard, C. Messier (1), O. Nibrat (1) and C. Destrade (1).

Lab. Neuroimmuno, IBON-CNRS, 35077 BORDEAUX and (1) Lab. Psychophysiologie, UA CNRS 399, 33405 TALENCE FRANCE.

This study examined the raising of rabbit polyclonal anti-idiotypic antiserum (AB2) that recognize dopamine (DA) receptors. Our rabbit polyclonal anti-idiotypic antibody (Ab) has consistated i (1) in developing polyclonal and monoclonal anti-DA conjugated antibodies which had high affinity for DA-S-cysteine, (11) in raising antibodies (Ab2) against these idiotypic antibodies (Ab), (11) Rabbit polyclonal Ab2 was obtained after alternate immunizations with either mouse Ab or rabbit polyclonal Ab with complete Freund's adjuvant. Then, the Ab2 were affinity chromatographed to remove anti-idotypic and anti-anti-idotypic antibodies. The specificity of the rabbit anti-idiotypic antibody was tested (1) in ELISA for their capacity to bind poly- and monoclonal idiotypic idiotypic sites, (2) for its ability to inhibit 3H DA binding to DA receptors; the 3H DA was displaced at 1/10000 dilution, (3) for its immunohistochemical visualization. Male rats were fixed with paraformaldehyde 4%picric acid 0.2%. Fifty sections were subjected to PAP staining, using a 1/1000 dilution of Ab2, Immunoreactive product was observed in striatum, septum and substantia nigra and was abolished after preadsorption with monoclonal or polyclonal Ab2. (4) Unilateral intra-accumbens injection of the anti-idiotypic in amphetamine pre-treated mice produced ipsilateral circling.

434.5 ROTATIONAL BEHAVIOR PRODUCED BY INTRA-ACCUMBENS MICROINJECTION OF CONJUGATED DOPAMINE ANTI-IDIoTypic ANTIBODY. O. Nibrat, C. Messier, W. Nofs (1), W. Geffard (1) and C. Destrade (1,2). (1) IBON: European Brain and Behavior Society.

Lab. Psychophysiologie, UA CNRS 399, TALENCE FRANCE and (1) Lab. Neuroimmuno, IBON-CNRS, BORDEAUX FRANCE.

Polyclonal dopamine (DA) anti-idiotypic antibodies were raised in immunized rabbits with powerful reactivity to various immunoglobulins conjugated nonconjugated DA antibodies (Chagnaud et al., J.of Neurochem., 40, 487-494, 1984). Antidopa antibody affinity and specificity were evaluated. In the present experiments, we tested the ability of the DA anti-idiotypic (AI) antibodies to change behavior through its action on a brain area rich in DA receptors, we microinjected DA AI antibodies into the olfactory bulb of adult rats, into the olfactory bulb of adult rats, into the olfactory nucleus accumbens of 2 mg/kg amphetamine treated BALB/c mice. One group was injected with either 0.5, 1.5 or 3.5 µl of DA AI; a second group injected with 3.5 µl of immunoglobulins from non-immune mice (fg); one group was injected with 1.5 µl of DA AI followed 45 min later by another injection of 3.5 µl of immunoglobulins from non-immune mice (fg); one group was injected with 1.5 µl of DA AI followed 45 min later by another injection of 3.5 µl of immunoglobulins from non-immune mice (fg); one group was injected with 1.5 µl of DA AI followed 45 min later by another injection of 3.5 µl of immunoglobulins from non-immune mice (fg). The injection of DA AI produced a locomotion which resulted in ipsilateral turning. No locomotion bias was observed in the mice injected with either of the fg doses. Injection of 1.5 µl of DA AI produced ipsilateral turning which was abolished and then reversed (contralateral turning) by the intra-accumbens somatodendritic injection of DA AI.
DOPAMINE RECEPTORS IV

434.7 COMPLEX DOPAMINE (DA) AGONIST/ANTAGONIST EFFECTS ON SUBSTAN-
TIA NIGRA PARTECULATA (SNpc) NEURONS. S.Martin Jr. and J.L.

Previous studies suggested that the ability of DA to in-
crease firing and to lessen responses of SNpr neurons to GABA could be mediated by D1 and D2 receptors, respective-
ly. Extracellular recordings were carried out in rats to ex-
amine the in vivo sensitivity of these receptors by iontophoretic application (2 and 5 nA) of either (-)-jujulgipride (SUL; 0.2M) or nortetidine (EST; 0.2M), both selective D2 antagonists. SKF 38393 inhibited SNpr firing with LY to further depress responses to GABA (n=7 cells). A similar pattern was also observed for (-)SUL (0.2M; 5nA) at 2 and 5 nA, and with (+)-SUL (5nA) could, however, block slowing of SN DA cell firing by LY. At 2 and 5 nA, (+)-SUL was also effective in increasing both synaptic and postsynaptic DA receptors.

434.8 EFFECTS OF SELECTIVE D1 AND D2 RECEPTOR AGONISTS ON THE
Res. Ctr, McGill Univ., Montreal, Canada, N4H 3M3 and Dept.

The effects of the selective dopamine (DA) D1 and D2 receptor agonists, SKF38393 and NO434.7 respectively, on the firing rate of medium spiny neurons (MSNs) were studied in young (3-5 months old) Fischer-344 rats. Multi-
barrel glass microprobes, filled with 1 M SKF38393 and NO434.7, were lowered into the anterodorsal cortical terminal field of the mesocortical DA system in urethane anesthe-
ized animals. The drug solutions were locally applied by pressure ejection. Both drugs produced dose-dependent and reversible reductions in firing rates. However, the D2 agonist was approximately 10 times more potent than the D1 agonist in suppressing the firing rate. The ED50 for NO434.7 and SKF38393 were 9.9 and 118.3 pS-sec respectively. Moreover, even at the highest doses, SKF38393 rarely produced complete cessation of firing in MPFC cells. Finally no evidence of synergism was observed when the two drugs were simultaneously applied; the effects of one drug were not potentiated by the concurrent application of the other. The present data suggest that D2 agonists are far more potent than D1 agonists in MPFC.

Supported by NSERC of Canada grant 19907392 and USDA grants 924418 and 9209199.

434.9 D1 SELECTIVE AGONIST SKF 38393 CAN ACTIVATE
STRIATAL NEURONS IN 6-HYDROXYDOPAMINE (6-OHDA)-
LESIONED RATS. R.G. Weick and J.R. Walters. NINCDS, Bethesda, MD 20892.

In rats with unilateral 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal dopamine (DA) pathway, some evidence suggests DA agonists may activate an inhibitory striatal input to the substantia nigra pars reticulata (SNr). Behavioral studies with these rats have attributed contralateral turning induced by DA agonists to increased GABA-mediated inhibition of SNpr cells. It has also been shown that i.v. apomorphine and the D1 agonist SKF 38393 inhibit SNpr single unit activity and markedly increase glucose utilization in these rats. We have utilized extracellular single unit recording techniques to compare, in anesthetized, colliculomotor, artificially respirated rats 6-8 weeks after 6-OHDA nigrostriatal lesion to examine whether i.v. SKF 38393 administration could activate the SNpr. Increases in the firing rates of striatal neurons with 6-OHDA lesions suggest the hypothesis that the population of striatongigantiglial inhibitory neurons has been activated by systemic administration of a D1 agonist in rats with 6-OHDA lesions.

434.10 DI DOPAMINE RECEPTOR STIMULATION ENHANCES THE POSTSYNAPTIC
BUT NOT AUTORECEPTOR EFFECTS OF D2 DOPAMINE (DA)
LEVER INTAKE IN RATS. B.G. Weick and J.R. Walters. NINCDS, Bethesda, MD 20892.

Recent electrophysiological evidence suggests that D1 dopamine (DA) receptor stimulation enhances the inhibitory effects of postsynaptic D2 DA receptor stimulation in the medial forebrain bundle (MFB). We have confirmed that the selectivity of these actions. Surprisingly, iontophoretic application (2 and 5 nA) of neither (-)-sulpiride (SUL; 0.2M) nor zetidoline (ZET; 0.2M), both selective D2 antagonists, could prevent the modulation of GABA effects by SKF38393. In addition, 3 of 5 quiescent neurons antidromically activated from the nigra began to fire after 3.4 mg/kg SKF38393.

434.11 NEUROPHYSIOLOGICAL EFFECTS OF (+)-UH 232 and (+)-AJ 76
ON DOPAMINE AUTORECEPTOR ANTAGONISTS, ON DOPAMINE
PRE- AND POSTSYNAPTIC RECEPTORS. D.A. Bergstrom, M.
Benito and J.R. Walters. NINCDS, Bethesda, MD 20892.

(+)-UH 232 and (+)-AJ 76 exert behavioral and biochemical effects which suggest that they can act preferentially at dopamine (DA) autoreceptors as antagonists (Svensson et al., 1996). Extracellular single unit recording techniques were used to evaluate the neurophysiological effects of these drugs to interact with nigra DA autoreceptors and postsynaptic DA receptors in the basal ganglia by comparing responses in GABAergic, presynaptic DA neurons and globus pallidus (GP) neurons, respectively, in locally anesthetized, colliculomotor, artificially respirated rats. At 50 nA, both drugs decreased GP cell firing but no changes were observed in SNpr dopaminergic neurons. (+)-AJ 76, however, increased the ED50 for apomorphine (AP)-induced inhibition of DA cell activity by 10-70 fold (control ED50 102±45 nA; +AJ 76 27±5 nA). Increases in GP cell activity induced by 0.3 mg/kg APO were effectively blocked by pretreatment or antagonists with 13 μmol/kg UH 232 (n=6) or AJ 76 (n=7). A 10- fold lower dose of (+)-UH 232 (13 μmol/kg) increased GP cell activity by 27±6% (n=7), increased APED50, decreased (-)-SUL 16±36 μg/kg (n=6) and effectively reversed AP-induced increases in GP cell activity. Thus (+)-UH 232 dose-dependent increases in the GP cell response to (-)-SUL and (+)-AJ 76 and demonstrated antagonistic effects both at DA autoreceptors and of postsynaptic receptors.

434.12 PARTIAL RECEPTOR INACTIVATION SHOWS LOW EFFICACY
OF S(+)-NPA. B.L. Vasquez and J.R. Winters, Deps. Pharmaco-
logical and Psychotherapeutics, Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

Previous studies showed that S(+)-NPA had dopamine (DA) agonist potency 300-fold lower than R(-)-NPA in slowing firing of nigral (SN) DA neurons. Antagonist effects were also evident since pretreatment with 40 μg/kg S(+)-NPA caused a significant rightward shift on the dose-response curve of (-)-NPA and R(-)-NPA, after partial irreversible inactivation of DA receptors with 60 μg/kg EEDQ reduced the maximal response to S(+)-NPA by 22% with a small shift on the dose response curve. The present data suggest that D2 agonists are far more potent than D1 agonists in MPFC.

Supported by NSERC of Canada grant 19907392 and USDA grants 924418 and 9209199.

434.13 PARTIAL RECEPTOR INACTIVATION SHOWS LOW EFFICACY

Previous studies showed that S(+)-NPA had dopamine (DA) agonist potency 300-fold lower than R(-)-NPA in slowing firing of nigral (SN) DA neurons. Antagonist effects were also evident since pretreatment with 40 μg/kg S(+)-NPA caused a significant rightward shift on the dose-response curve. The present data suggest that D2 agonists are far more potent than D1 agonists in MPFC.

Supported by NSERC of Canada grant 19907392 and USDA grants 924418 and 9209199.
435.1

REGIONAL DIFFERENCES IN THE BINDING OF PIRENZEPINE AND AF-DX 116 TO RAT BRAIN. COMPARISON WITH MINIMUM ENERGY CONFORMATIONS. W. Lim, B.R. Ellinbrock*, D.A. Smith and W.A. Meager, Jr. Dept. of Medicinal and Biological Chemistry, and Dept. of Chemistry, Univ. of Toledo, 2801 W. Bancroft St. Toledo, OH 43606.

The binding of selective muscarinic receptor antagonists to regions of rat brain was examined through autoradiographic techniques. Pirenzepine and AF-DX 116 were chosen because of their selectivity for M1 and M2 muscarinic receptors respectively, and similarities in chemical structure. Pirenzepine displayed a higher potency than AF-DX 116 for the inhibition of [3H]-QNB-labeled binding to rat brain sections. Analyses of binding to brain sections revealed heterogeneous binding profiles for both antagonists, suggesting the presence of multiple receptor sites.

Quantitative data were derived from regional analyses of pirenzepine and AF-DX 116 binding. Pirenzepine displayed the highest affinity for hippocampal, striatal, and amygdaloid muscarinic receptors (IC50s < 0.4 µM). M2 receptors displayed a slightly lower affinity for cortical receptors (IC50s between 0.4 and 0.8 µM). Pirenzepine displayed the highest affinity for thalamic and brainstem regions (IC50s generally > 1.0 µM). In contrast, AF-DX 116 bound with higher affinity to hippocampal, striatal, and amygdaloid muscarinic receptors (IC50s < 0.5 µM) than to receptors in thalamic and brainstem regions (IC50s > 1.0 µM). Binding sites with the lowest affinity for AF-DX 116 were found in cortical, striatal, and hippocampal regions (IC50s > 2.0 µM). The binding profiles of the two selective muscarinic antagonists reveal the complexity and diversity of muscarinic receptor subtypes throughout the brain. The data provide a basis for identifying muscarinic receptor subtypes with selective ligands.

Minimum energy conformations of pirenzepine and AF-DX 116 were calculated using the program MacroModel (version 1.5). Pirenzepine displayed three energy minima, differing in the relative position of the piperazine ring with the respect to the tricyclic system. In contrast, the diethylaminoethyl substituent on the piperidine ring conferred a much larger set of minimum energy conformations on AF-DX 116. It is suggested that the defined conformations of pirenzepine allow it to achieve a conformation accessible to pirenzepine that can bind to M2 receptors. Supported by NS 29329.

435.3


M1 and M2 muscarinic receptors mediating separate biochemical effects are found in the rat neuroblastoma clone N1E-115. The M1 receptor elicits cAMP, while the M2 receptor inhibits PGF1-elevated cAMP formation. We used these two antagonists to screen several muscarinic antagonists. An 'equivalent molar ratio' (EMR=IC50/pIC50 of antagonist) was calculated for each of twelve antagonists, with the EMR being used to compare their selectivity for blockade of carbachol-activated M1 and M2 responses. Pirenzepine was 30-fold M1-selective, in agreement with selectivity found by Schild analysis (McKinney et al., Mol. Pharmacol. 27:223, 1985). Several other antagonists were M1 selective: trihexyphenidyl (26-fold), bezapirone (2-fold), and benzhexol (3-fold). Four antagonists were M2-selective in this comparison; purnacurin (7-fold), scopolamine (10-fold), AF-DX 116 (4-fold), and 4-UMAP (3-fold). Central M1 and M2 receptor binding potencies were measured by blockade of ['3H]pirenzepine binding in the rat cortex and by blockade of ['3H]AF-DX 116 binding in the striatum and pons, respectively.

Interesting similarities and differences arose when functional selectivities were compared to those determined by binding potencies.

435.5


The use of selective muscarinic antagonists in radioligand binding studies has greatly enhanced our knowledge of muscarinic receptor heterogeneity. We extend these studies through the characterization and autoradiographic depiction of [3H]AF-DX 116 binding sites in the rat CNS. Incubations for all experiments were in Krebs buffer at 25°C for 60 min. Significant reductions in [3H]AF-DX 116 binding were noted in many CNS areas when 18 month old rats were compared to 3 month old controls. The cerebral cortex showed the most substantial decreases in binding. When 18 month old rats were compared to 3 month old controls, the cerebral cortex showed the most substantial decrease in binding capacity (over 20 percent) were noted in many CNS areas.

Data obtained by the use of selective antagonists such as pirenzepine (PZ) and AF-DX 116 are shown to the subtypes of muscarinic receptors. Pirenzepine inhibited the acetylcholine-stimulated release of catecholamines from isolated, perfused guinea pig adrenal glands in a dose-dependent manner. This inhibition was not observed by perfusing the tissue with Locke's solution and was not due to a non-selective alkylation by the isothiocyanate function. This preliminary data suggests that pirenzepine may be binding to muscarinic receptors in an irreversible manner.

435.6


The development of the ligands has proven to be an invaluable technique for the characterization, isolation and purification of these receptor systems. The preliminary determination of an isothiocyanato-derivative of aprontiflorin (aporphine) as a potential irreversible antagonist of muscarinic receptors in the rat brain. This inhibition was not observed by perfusing the tissue with Locke's solution and was not due to a non-selective alkylation by the isothiocyanate function. This preliminary data suggests that PZ may be binding to muscarinic receptors in an irreversible manner.


The coupling to biochemical effector systems of central M1 receptors (cortical phosphoinositol metabolism) and M2 receptors (cortical and striatal forskolin) is studied in metabolically-prelabeled, mechanically-dissected cellule preparations of the adult rat brain. Cortical [3H]inositol was used to measure the activity of the muscarinic receptor with high affinity by pirenzepine with high affinity (K=1.0 µM) while this antagonist blocked cortical and striatal forskolin- elevated [3H]cAMP formation with K= values of 354 µM and 325 µM, respectively, indicating coupling of the latter response to M1 receptor. Proprylene glycol mustard (IC50=6 µM) was employed to partially occlude muscarinic receptors in this preparation and the equilibrium binding constants for carbachol and M1 and M2 responses were determined. Carbachol was bound to the M1 receptor with a Kd value identical to its EC50 (104 µM), while this agonist mediated the M1 response in control experiments with Kd values of 15 µM and 2.0 µM. These findings indicate that the central M1 receptor is activated by the agonist binding in a low-affinity agonist-receptor conformation, while the central M2 receptor is activated by the agonist in a high-affinity active conformation.
CHARACTERIZATION OF MUSCARINIC RECEPTORS II

435.7
DEVELOPMENT OF M1 MUSCARINIC RECEPTORS IN FETAL AND NEONATAL MOUSE BRAIN AND HEART: J.-C. Anzil*, M. Nan*(*), H. Yamasaki*, H. Yamasaki, and M.R. Buehler. Deps. of Pharmacology and Internal Medicine, Univ. of Arizona, Col. of Med. Tucson, AZ 85724.

Development of M1 muscarinic receptor (mChRs) has been studied in the fetal, neonatal and adult CD-1 mouse brain and heart. mChRs were detected using [3H]quinuclidinyl benzilate ([3H]QNB) and the M1 receptors determined using the selective ligand [3H]AF-DX 116. The total mChRs and the M1 receptors in both tissues reached their adult level after 42 postnatal days and half maximal value at about 14-21 postnatal days. The concentrations of total brain mChRs and the M1 receptors, calculated on the basis of either protein or tissue contents, increased continuously from fetal to postnatal periods. In contrast, the heart mChR concentrations, based on either protein or tissue contents, reached the adult level as early as at the birth. The concentration of heart mChRs kept increasing after birth with a peak level at 14 postnatal days and dropped down to a lower level in the adult, suggesting a preferential development of mChRs during early postnatal period. The percentage of M1 receptors vs total mChRs was constant (16-26%) in the brain while it varied (45-70%) in the heart during the development. In conclusion, the murine neuronal and cardiac M1 receptors showed differential patterns of development.

435.8
EFFECT OF METHOCTRAMINE ON MUSCARINIC RECEPTORS IN MURINE NEUROBLASTOMA CELLS: A.D. Frieser*†, N.L. Lee, E.E. El-Fakahany (SPON: N.Hazen). Dept. of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Methoctramine (Met) is an antagonist with greatest affinity for cardiac M2 muscarinic receptors which also discriminates between muscarinic receptors in the cortex (M1) and glands (glandular M2) (E.J. 1988, 152, 1-11). Muscarinic effects were examined using NIE-115 mouse neuroblastoma cells (NB cells). NB cells have 2 muscarinic receptor subtypes, M1 receptors which mediate phosphoinositol turnover (PI) and M2 receptors which inhibit cyclic AMP (cAMP) formation. Calculations were made under the assumption that Met is a competitive antagonist. The dissociation constant at the doses used did not alter the rate of dissociation of bound [3H]pirenzepine (PZ) from the receptors. Met displaced [3H]QNB (0.2 mM) binding to one site in NB cells (Kd of 120 ± 11 nM, ng close to 1.0, data showed best fit to one site using LIGAND). Met was also an equipotent antagonist for PI (stimulated by 1.0 mM carbamylcholine (CCh)) and cAMP responses (measured with 25 mM forskolin in the presence and presence of 0.1 mM CCh). The Kp values were 132 ± 19 and 155 ± 25 nM respectively and the ng were close to 1.0. Thus, in NB NIE-115 cells, Met does not show the obvious selectivity for muscarinic receptor subtypes reported using other tissues. (Supported in part by NH grants 1FR2HL7691-01 CLN, NS-24158, AG-07118, AG-00344.)

Gallamine and other compounds (verapamil, flunarizine, and the benzodiazepine diazepam) were found to inhibit [3H]QNB binding to rat brain homogenates, and to inhibit [3H]QNB binding to membranes isolated from rat brain. The affinity and intrinsic activity of several acetylcholinesterase inhibitors (ACHEIs) against [3H]QNB and [3H]CMD binding were determined in the presence and absence of the competitive antagonist dihydro-2-chloroquinuclidinyl benzilate ((3H)NMeQNB). The affinity and intrinsic activity of several AChEIs against [3H]QNB binding correlated with their potency to inhibit QNB/CMD ratio for the competitive agonist arecoline was not significantly affected. These results suggested that for those AChEIs examined the displacement of [3H]QNB binding was due to a direct interaction at the receptor. In addition, using this two assay binding method, PAR pretreatment shifted QNB/CMD values for these AChEIs toward 1 due to a decrease in potency to inhibit [3H]QNB binding; whereas, the QNB/CMD ratio for the competitive agonist arecoline was significantly affected. Therefore: [3H]QNB binding was due to a direct interaction at the receptor.

MUSCARINIC RECEPTORS John E. Ellis. Neuroscience Research Unit, Department of Psychiatry, University of Vermont (BSRG).

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Despite the known neurotoxic effects of lead, its locus of action is unclear. We have investigated the effects of lead on calcium currents of Aplysia neurons recorded using a two-electrode voltage clamp technique. The threshold concentration for effect was 1 μM lead. At 5 μM lead, reduced the peak amplitude of the calcium current by 14.7 ± 5%, at 10 μM by 26.3 ± 2.6% and at 50 μM by 57.7 ± 4%. With washing the calcium current amplitude rapidly (within 2 to 3 min) returned to control levels. The mechanism of these effects is not understood. Nonspecific effects caused by lower fluid consumption. No lead doses caused changes in sodium, calcium, or potassium channel currents. Partial improvement of the toxic effect of aluminum administration and that it can be reversed by administration of an aluminum chelator.

Studies have shown an effect of Pb on neurophysiological function and an interaction of zinc (Zn) with Pb. We have evaluated the effects of low level Pb and its interactions with dietary Zn in 3 groups of immature guinea pigs. Group 1 received standard guinea pig chow (27 ppm Zn); group 2 received egg white based diet (5 ppm Zn); and group 3 received a diet of egg white and 50 ppm Zn. One third of group 1 received 10 mg Pb acetate/kg body weight and one third received 20 mg Pb acetate/kg body weight. Half of groups 2 and 3 received Pb acetate/kg body weight.

In both Pb-treated and control groups, the mean maximum diameters of the astrogia were increased in the Pb-treated animals. Neither Pb nor Zn had an effect on absolute brain weight or brain weight to body weight ratios. Dietary Zn did not affect levels of Cu, Zn, or Pb in the brain. Further evaluation of the significance of an increase in estragioal diameter without concentration of Pb in the cerebrum is underway. Funded by Center for Energy & Mineral Resources, TAMU and EPA R811500.

**REFERENCES**


**FREE AMINO ACIDS IN PLASMA AND BRAIN AFTER CHRONIC MANGANESE INTAKE.**


The present study was conducted to determine the changes in the concentrations of free amino acids in plasma and brain after chronic manganese intake.

- Male Sprague-Dawley rats weighing 200-250 g were treated with 1 μg of Mn per ml of drinking water. Both the manganese-treated and the control group had unrestricted water access. At eighth month, all animals of each group were sacrificed by decapitation. The brains were extracted immediately and placed on ice to extract and freeze. Prior to the sacrifice a blood sample was obtained from each rat by cardiac puncture. The analysis of the amino acids was performed by HPLC.

The growth rate of manganese intoxicated rats and the brain manganese concentrations increased significantly. The means of the manganese content (expressed in μg/dry weight) at the eighth month were: a) frontal cortex: 0.7 ± 0.3 in controls and 5.1 ± 0.2 in Mn-loaded rats; b) striatum: 1.8 ± 0.3 and 3.2 ± 0.2. The mean ± S.E. daily water intake during the eighth month for each rat was: 80.1 ± 0.7 ml for controls and 54.0 ± 0.5 for the Mn-treated rats. The difference was significant (p < 0.001).

No changes were observed in the levels of plasma amino acids in manganese-treated rats. Small results were observed in the striatum and frontal cortex. In the light of these results it is safe to assume that the chronic manganese intake of 1 mg Mn per ml of drinking water did not affect either the gastrointestinal absorption of the amino acids nor their passage through the blood-brain barrier.
346.7 MECHANISM OF TRIETHYLTIN BROMIDE-INDUCED NEUROTOXICITY. B.E. Morton, J.P. Fishbein and P. Zakeri, Children's Hosp. of Milwaukee, Milwaukee, WI 53226. Respiratory is diminished in brain slices from rats injected with triethyltin bromide (Fischer J. 119 95-102, 1970). Pyruvate dehydrogenase activity is also reduced in brain homogenates or mitochondria prepared from TET-intoxicated rats (FASEB J. 2 A 1373, 1988). We have now investigated whether TET affects glucose metabolism in 11ve, conscious rats by analyzing its influence on the uptake of 2-deoxyglucose (2-DG) in brain (14C)-2-DG (125UCI/Kg) was injected i.p. at timed intervals after i.p. injection of TET into groups of rats. Animals were sacrificed 45 min later and their brains removed for sectioning and autoradiographic analysis (J. Neurochem, 78 897-916, 1977). In addition, we investigated possible mechanisms by which TET may inhibit neurotransmitter receptor agonists and antagonists as potential antidotes for TET-neurotoxicity. Ligands, acting on ionophore-regulating inhibitory and excitatory receptors were administered by i.p. injection prior to TET administration and their influence on the course of development of neurotoxicity was monitored. Statistically significant reductions in global and regional uptake of 2-DG were found in brains of TET-treated rats compared with control animals. Administration of the putative antidotes resulted in complex changes suggesting that some of the effects of TET can be modified by ionophore regulation. [Supported by grants from the NIH (ES00405) and The Univ. of Hawaii Foundation.]

346.8 TRIETHYLTIN NEUROTOXICITY IS NOT MEDIATED BY EXTRA-CELLULAR CA**+**: F.-J. Fournier et al., Neurotoxicology, Teratol., in press), we exposed in vitro hippocampal slices to TET in a Ca**+-free environment. Slices were prepared using standard techniques and maintained at the interface of an artificial CSF and an O2/CO2 atmosphere. Recording and stimulating electrodes were positioned in the Schaffer collateral. When a stable waveform was recorded, a series of increasing stimulus intensities was employed. Following the baseline 1/0, Ca**+ was removed from the bath by removing the EEP. After 1 hr, the EEP could no longer be evoked, and at this time any tissue-bound Ca**+ released after the first exchange was removed with a second Ca**+-free washout. Slices were exposed to 10 nM TET. Evoked responses were recorded for 2 hrs at 5 min intervals, only to be interrupted at 1 and 2 hr post-exchange for assessments. After the 1/0 assessment, at 2 hr post-exposure, was washed out of the pool using Ca**+-free, medium, followed by an exchange of Ca**+-bearing medium. Ca**+-free medium did not produce recovery of the EEP. Thus, TET-induced synaptic depression is probably not mediated through extracellular Ca**+. It is possible that attenuation of evoked synaptic responses reflects TET-induced gradual depression of the synapse by uncoupling oxidative phosphorylation. [Supported by EPA, ONR and NIH]


Previous immunocytochemical and histochemical studies have indicated a specific reduction in GFAP in human hepatic encephalopathy (HE). Since ammonia is a prime candidate as the etiologic factor in HE and since astrocytes are affected in HE, we studied the effect of ammonia on the BZD receptor in primary astrocyte cultures isolated from neonatal rat cortex, and after 2 weeks, half of the cultures were treated with 0.5 mM dibutyl cyclic AMP (dBcAMP). Scatchard analysis of the binding of [3H]-BZD to astrocytes homogenates in the presence of 2 and 5 mM NH4Cl showed a significant increase in Kd (18.1 ± 4.6, p<0.01) and 32%, respectively (p<0.05) in cells that had not been maintained with dBcAMP. However, no significant increase in binding affinity was observed in the presence of 10 mM NH4Cl. No significant change in Kd nor Bmax was found at any NH4Cl concentration. Dibutyl cyclic AMP (100 M) decreased Kd in control cultures (4.5 ± 1.2 10-9 M) to 2.5 ± 1.2 10-9 M while Bmax was increased from 1.1 ± 0.2 pmol/mg. The latter results are consistent with the observation that dBcAMP has a protective effect on the ammonia-induced morphological changes observed in cultured astrocytes. Our findings furthermore suggest that some of the effects on BZD receptors may occur on astrocytes and thus implicate the astrocyte BZD receptor in the pathogenesis of hepatic encephalopathy.
Hydrogen sulfide (H2S) and its alkali salts are very toxic. H2S has caused many industrial deaths, apparently by paralysis of respiratory drive. Few electrophysiological studies of its toxicity have been done (Warenycia et al., Soc. Neurosci. Abstr. 13, 1987). The effects of NaHS on neural control of respiration, regional CA levels were activated hyperpolarization), and on agonist-induced conductance changes in sympathetic ganglia of Rana pipiens. Muscarine-induced (10 µM) hyperpolarization was potentiated to 108.8 ± 5.2% of control (p<0.05, n=7), but removal of NaHS potentiated the response. A consistent NaHS-induced depolarization was observed (2.75 ± 0.5 mV, p<0.001, n=23). The lack of significant attenuation of these diverse responses does not support a rapid anti-metabolic action of NaHS. Supported by Alberta Community and Occupational Health.

**ALZHEIMER’S DISEASE: NEUROPATHOLOGY**


The distribution of cells containing copper zinc superoxide dismutase (CuZnSOD) protein and mRNA was determined using immunohistochemistry and in situ hybridization in hippocampi and in various cortical regions from control human brain. Results obtained with these two methods are similar and demonstrate that pyramidal neurons and granule cells of hippocampus contain high amounts of CuZnSOD protein and mRNA. In the hippocampus of an Alzheimer’s patient, surviving cortical neurons were immunostained by antiCuZnSOD and antipaired helical filaments (PHF) antibodies show that amyloid plaques are distributed in a pattern that is consistent with the pathologic pattern of Alzheimer’s disease. This might indicate that biochemical pathways leading to O2- and HO2- production may be facilitated in these neurons, requiring a high CuZnSOD content to eliminate these radicals.

Quantitative analysis by in situ hybridization are in progress to evaluate the transcription of CuZnSOD gene in Alzheimer’s disease brain.

437.2 A REEXAMINATION OF ALUMINUM IN ALZHEIMER’S DISEASE. R. W. Jacobs, T. Duong, P. E. Jones* and A. B. Schelbe.

This study reassesses the presence of aluminum in Alzheimer’s disease (AD) as previously reported by Perl and Brody (1984) and Candy et al. (1986) using X-ray microprobe analysis. Hippocampal and neocortical tissue samples were obtained from five clinically diagnosed neuropsychologically confirmed cases of AD (3 females/2 males; ages 74-102 yrs) and five controls (1 female/4 males; ages 43-74 yrs) with autolysis times between 7 and 30 hrs (mean of 13.5). To demonstrate senile plaques and neurofibrillary tangles, alternating cryostat sections (40 µm) were treated with thioflavin-S, Congo red, Bielschowsky’s silver stain, and by immunohistochemistry for amyloid. Cryostat sections were microprobed. Virtually no aluminum or aluminosilicates were found in the AD cases or in the controls. There was also no unique distribution pattern or “signature” of chemical elements to distinguish the hallmark lesions of AD. We conclude that aluminum does not appear to play a significant role in the manifestation of AD. Further investigations should settle this important question.
ALZHEIMER'S DISEASE: NEUROPATHOLOGY

THURSDAY PM

STELLATE CELLS IN LAYER II OF THE ENTORHINAL CORTEX ARE PRONE TO NEUROFIBRILLARY TANGLE FORMATION IN AGING AND ALZHEIMER'S DISEASE. T. Dzung, T. Abele, and A.B. Scheibel. UCLA Departments of Anatomy, Psychiatry and the Brain Research Institute, Los Angeles, California 90024.

We have studied the layer II neurons in the entorhinal cortex of Alzheimer's (5 female / 5 male; age = 66 - 102 years) and normal patients (5 male; age = 61 - 75 years). All clinical diagnoses of Alzheimer's were neuropathologically confirmed. The postmortem time varied from 7 to 15.5 hours. Cryostat sections (40 µm) were processed by routine histological stains and peroxidase-antiperoxidase immunocytochemistry directed against human amyloid precursor protein (Ab 42), the 45 kDa catalytic domain of PKC, the 35 kDa regulatory domain of PKC, and the 82 kDa PKC isoform. The presence of these proteins was assessed in neurons and in neurofibrillary tangles (NFTs) using immunoblotting experiments, immunoprecipitation experiments, and Western blotting. The results indicate that the layer II neurons in the entorhinal cortex are more susceptible to neurofibrillary tangle formation than other neurons in the brain. These results suggest that the layer II neurons may be a preclinical or early stage of Alzheimer's disease (AD), and that they could be a target for therapeutic intervention.

SITES OF EARLY ALZHEIMER-TYPE PATHOLOGICAL CHANGES IN ENTRORHINAL CORTEX AND HIPPOCAMPUS VISUALIZED BY ALZ-50 IMMUNOCYTOCHEMISTRY. B.T. Hvas and G.W. Van Hoye. Dept. of Neurology, and Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The presence of a few neurofibrillary tangles (NFTs) in the brain of a "normal" elderly person is an unusual pathological finding. These lesions may represent a preclinical or early stage of Alzheimer's disease (AD), and it is possible that the sites of AD pathology in these patients are the initial lesions of the disease. To explore this, we have selected for cases, regardless of clinical history, in which minimal or no NFTs were present. The topography of pathological lesions was noted using both thioflavine S and Alz-50 immunocytochemistry, an antibody that recognizes NFTs of the "alpha-NFT" stage. In these cases, NFTs and Alz-50-positive neurons were found in layer II of the entorhinal cortex, the continuation of this layer into layer III of parahippocampal cortex, and the subicular/CA1 area. This suggests that these lesions could be an early indication of Alz-50 pathology.


Morphological studies of the brain of patients with Alzheimer's Disease (AD) have reported significant changes in the neuron number and the dendritic structure of cortical neurons. These data, obtained from autopsy material, suggest an decrement in the number of synaptic contacts as a consequence of the disease. The present study examined the density of synaptic contacts in the frontal cortex (Brodmann's area 9) from autopsy-proven AD patients and age-matched, post-mortem matched controls taken within 13 hours of death. The brain tissue was prepared for ultrastructural analysis using immersion fixation and standard osmium tetroxide postfixation. Stereological methods were employed to ascertain synaptic density in cortical lamina III and V. Individuals with AD exhibited a highly significant reduction in synaptic density in both laminae as compared to controls. Mean synaptic apposition length was significantly larger in AD patients and was highly correlated with individual synaptic density in both control and AD brain. This suggests a possible compensatory mechanism. These results could not be accounted for by differences in postmortem interval or by the mean age of the groups. Coupled with the reported decline of ascending (extrinsic) cortical inputs and concomitant intrinsic neuronal loss, these results indicate that AD cortex is unlikely to maintain normal synaptic density and shows a diminished regenerative response. (Supported by ADRDA IRG-87042, NIH AG03519 and NS21541 and the VA Research Service.)


We have studied the layer II neurons in the entorhinal cortex of patients diagnosed with Alzheimer's disease (AD). The findings suggest that these neurons are vulnerable to Alzheimer pathologic degeneration, and may be among the first sites of pathology in the brain. (We thank V. Davies for generously providing Alz-50; supported by the Makers Foundation, and NS 18444 and PO NS 19032.)


Pathological hallmarks of AD, plaques, tangles, and specific cholinergic neuronal loss, have not indicated any unusual cell surface dysfunctions. This is curious in view of the amyloid precursor gene encodes a polypeptide with a transmembrane spanning sequence. We are studying neuronal surface molecules utilizing monoclonal antibodies made to cholinergic nerve terminals. The antibody Tor-23 binds to a determinant membrane of a discrete population of neurons in the human cortex and a subpopulation of subcortical astrocytes (Stephenson et al., these abstracts). In this study we examined cortical and subcortical regions from AD cases to determine if the epitope defined by Tor-23 was present, absent, or altered. Examination of the cortex from six AD cases by immunohistochemical analyses revealed distinct differences in the distribution of Tor-23 binding relative to that of control cortex. In AD, there were fewer Tor-23 positive neurons and the immunopositive astrocyte was absent. Because a particular epitope was localized, we cannot be certain that cells were lost; they could have lost immunoreactivity. Given its pivotal surface location in the adrenergic species, the fallout of the epitope alone may be significant. Because reactive gliosis is present in AD cortex, our finding of the loss or transformation of a significant cell line is of further examination. Funded by the Department of Health Services, California.
437.9


The present in situ findings within the iso- and allocortices support the distribution of Tορ 23 to the apparent limiting membrane of rare and select neurons. In the frontal, occipital, parietal and motor cortical areas, Tορ 23 outlined neurons of laminae 2-3. These neurons were medium to large sized, rounded, and non-pyramidal. In the hippocampus, immunopositive neurons were large and were usually located just external to the pyramidal cell layer. In addition to the stained neurons, Tορ 23 bound astrocytic cells of the subcortical white matter. Co-staining with GFAP indicated that only some subcortical astrocytes were Tορ 23 positive. The antigen appears to be a polypeptide by immunoblot analysis.

The conservation of an epitope from elasmbranchs to humans is evidence in favor of an important function. Its position on the neuronal external surface suggests it may play a dynamic and vital role in developing and maintaining the complex neuronal milieu of the human cortex. Its appearance on a population of astrocytes may reflect a neuronal-glial interaction among a highly specialized neural subpopulation.

437.10


A new monoclonal antibody is described. Partially purified amyloid protein isolated from AD meninges was used as immunogen in mice. In formalin-fixed paraffin-embedded tissue sections from cases of AD, this antibody labels extracellular ("ghost") neurofibrillary tangles (NFT) but not intracellular NFT. Some amyloid plaques are also labeled, and this immunostaining is enhanced by pretreatment of tissue sections with 8% formic acid. Formic acid pretreatment does not alter labeling of extracellular NFT and does not cause intracellular NFT to become immunoreactive. On frozen sections of brain tissue, this antibody labels nuclei of all cells. This antibody does not recognize bovine tau protein or purified glial fibrillary acidic protein on Western blots, nor does it recognize the 28 residue synthetic beta amyloid peptide on dot blots. No protein bands are labeled on Western blots of Triton-soluble proteins from homogenates of AD or control cerebral cortex. Studies are under way to identify the antigen(s) recognized by this antibody. Our hypothesis is that proteins immunologically cross-reactive with the beta amyloid protein may coat the filaments of extracellular tangles, and thus be recognized by this antibody.
ALZHEIMER'S DISEASE: NEUROPATHOLOGY

THURSDAY PM

437.15
QUANTITATIVE ANALYSIS OF NON-PHOSPHORYLATED NEUROFIBRILLARY PROTEIN (NFP) IMMUNOREACTIVE NEURONS IN NORMAL AND ALZHEIMER'S DISEASE BRAIN. P.B. Huf, K. Cox*, and J.H. Morrison, Research Institute of Scripps Clinic, La Jolla, CA 92037.

We have postulated that pyramidal neurons that are prone to neurofibrillary tangle (NFT) formation in Alzheimer's disease (AD) contain a high somatic and dendritic density of NPNFP in the healthy aged cortex. In addition, the intracellular concentration of NPNFP is directly correlated with the cell size. In order to further investigate the relationship between cell size and intracellular concentration of NPNFP, and NFT formation, we performed a quantitative analysis of the distribution and density of NPNFP-immunoreactive neurons in the normal and AD neocortex. In the AD brains, these distribution patterns were correlated with those of plaques and NFT. The immunoreactive cells were divided into three size groups: < 250 µm², 250-350 µm², > 350 µm². In layer III, a significant (72%) loss was observed only in cells > 350 µm², whereas in layer V, there was a moderate decrease in the intermediate-sized cells (44%), and a dramatic loss in the large cell group (78%). Even though cell loss was only apparent among the larger neurons, a decrease in the intracellular concentration of NPNFP (as measured by optical density) was observed in all the three size groups. In all the AD cases, there was a high number of plaques and NFT in layers III and V. These data further support the hypothesis that NPNFP is particularly vulnerable in AD. Supported by grants from ADRDA, AG 06649 and NRSA 019-05.87.

437.16

We compared tau immunoreactive features to argentaffin features using the sensitive Gallyas intensified Hki procedure developed by Campbell et al (Soc Neurosci Abs 13:578) in the CA 1 field of the hippocampal formation of 5 patients with Alzheimer's disease (AD).

Tau histchemistry showed a plexus of dystrophic neurites (DN) spanning CA 1, with greatest density near the stratum oriens and radiatum borders. A band of typical neurofibrillary tangles (NFT) and occasional senile plaques (SP) were seen in the center of CA1. In contrast, the silver method marked NFT throughout CA1. These included typical NFT seen with tau histchemistry and a group of larger diffusely argentaffin tangles. DP were homogenus in distribution and longer than tau reactive DN. SP were more dense and formed a continuous band in stratum radiatum. In one case showing early changes of Alzheimer's disease, the silver method showed marked homogenously distributed SP only whereas tau histchemistry marked a perivascular location. In all cases, the plexus of dystrophic neurites showed a strong positive stain for tau. This work strongly suggests that the argentaffin composition of the tangles is an early marker of Alzheimer's disease.

437.17

A number of previous investigators have examined the laminar and regional distributions of neurofibrillary tangles (NFT) and neuritic plaques (NP) using small blocks of tissue sampled across different regions of Alzheimer (AD) brains (e.g., Leutet et al., 1987). While these studies have provided critical quantitative information, it has been difficult to conclusively determine if the spread of AD pathology in the brain cortex [CC] follows ortho- and retrograde routes. Our aim has been to develop computer-assisted visualization techniques that will enable us to systematically characterize the pathological distribution of NFT and NP throughout the entire extent of temporal and occipital cortices with the detailed connectivity patterns for these same regions. We believe these methods will enable us to trace the spatially consistent changes in distribution of AD pathology and cortical connectivity.


437.18

The emerging picture of Alzheimer's Disease (AD) as disruption of specific cortical and subcortical structures necessitates closer examination of subregions of primary limbic structures affected by the disease. Since the amygdala is a principle limbic structure heavily involved in both the behavioral and pathological manifestations, we studied various amygdaloid subregions for differential alterations in AD.

Three dimensional reconstructions of the entire amygdaloid complex were employed. Frozen 50 µm thick sections were obtained throughout the entire amygdala with every fifth section counterstained with cresyl violet.

The amygdala in subjects with AD demonstrates a striking topographical rearrangement and reduction in size. In AD the structure appears to have lost considerable volume, with secondary dilation of the inferior horn of the lateral ventricle (ex vacuo). The rostrocaudal extent of most nuclei, as well as the entire complex, is reduced. The nuclei reveal a distorted outline in relation to each other and to adjacent structures, most marked in the basolateral group. Volumetric changes in specific areas and nuclear groups will be discussed.

(Supported by ADRDA IIRG-87-042, NIH AG05119 & NS01541 and the VA Research Service.)

437.19
HLA-DR-POSITIVE MICROGLIA IN NORMAL AND DEMENTED BRAINS. L.A. Mattiske, G. Cooper* and D.W. Dickson*, Dept. Pathol., Albert Einstein College of Medicine, Bronx, NY 10461.

Several recent studies using monoclonal antibodies specific to HLA-DR, a class II major histocompatibility complex cell surface glycoprotein, have demonstrated HLA-DR on a small number of microglia in the normal central nervous system. For more HLA-DR-positive microglia have been described in Alzheimer's disease (AD), raising the possibility that immune mechanisms may be operative in AD. 

Brains were obtained at autopsies of 2.5 to 2.5 hours post-mortem from 14 individuals: 1 child, 2 young adults, 2 adults with neurologic disease, and 9 cases of AD (3 early, 6 chronic). Staining of microglia with antibodies to HLA-DR was highly sensitive to duration and type of fixation. Optimal staining was obtained with short duration (5 min.) fixation, cyropreservation with sucrose and vibratome sections. HLA-DR-positive neurons were seen in both gray and white matter of all cases. The exception of chronic AD, microglia with delicate, highly branched processes were uniformly distributed. In chronic AD, microglia, with short, stubby processes or rounded contours were clustered. Double-labeling with fibrillary neurofilament protein demonstrated that HLA-DR-positive cells were not astrocytes. With electron microscopy, labeled cells were consistent with microglia. In AD, they had prominent phagolysosomes. Double-staining with antibodies to beta-amyloid synthetic peptides showed microglia associated with amyloid deposits.

437.20
THE DISTRIBUTION AND DENSITY OF ALZHEIMER-LIKE NEUROPATHOLOGICAL MARKERS IN NON-DEMENTED AND MILDLY DEMENTED BRAINS. J.L. Price, D.L. White* and P. Davis*.

As part of an ongoing study, tangles, plaques and Aβ-50 immunoreactive cells (AS50) have been mapped in the brains of cognitively unimpaired and demented subjects, 59 to 87 years in age. The cognitive status of each case was assessed by premortem testing in the Memory and Aging Project of Washington Univ, or by a structured retrospective interview with a close relative. Serial sections through the ventral forebrain were stained with the Bielschowsky silver method, or immunohistochemically with the Alz-50 antibody, and then mapped and counted with the aid of a computerized microscope digitizer. To date 7 unimpaired cases, 3 very mildly demented cases and 4 severely demented cases have been studied.

In the unimpaired cases, the highest density of tangles and AS50 was found in the perirhinal cortex, followed (in order) by the entorhinal cortex, anterior olfactory nucleus, and hippocampal field CA1. A similar pattern was found in the mildly demented cases, although the overall density of these markers is greater. The pattern also appeared to be continued in the severely demented cases, but the high density of tangles and AS50 in many other brain regions was not observed.

These results support the concept that there is a continuum between healthy aging and Alzheimer's Disease.

However, the incidence and distribution of plaques is more selective. Almost no plaques were found in the unimpaired cases, except for one case which also had a high density of tangles, while all but one of the demented cases had a high density of plaques.

Supported by NIH grants AG05681 and NS09518.
ALZHEIMER'S DISEASE: NEUROPATHOLOGY


Maximal seizure spike (SF) density 15/15/mm² in neocortex meets the part II criteria for Alzheimer's disease (AD), but some non-demented individuals have both high SF counts and preserved choline acetyltransferase (ChAT) in neocortex. We examined these relations in a 76 year old non-demented man and extend previous work by analysis of acetylcholinesterase (AChE) isozymes. Microscopic exam showed abundant cortical SFs, occasionally 15/mm², and rare neurofibrillary tangles (NFTs) in frontal, parietal, and temporal cortices. Amygdala had highest SF counts. Despite sclerosis of CA1 region in hippocampus, neither SFs, NFTs, nor granulovacular degeneration were found in hippocampal gyrus, although subicular and parahippocampal gyrus had these abnormalities. No neuronal loss or NFTs were seen in basilar or septal nuclei. CHAT activity in hippocampus, septum, and parietal cortex was similar to 3 aged control and higher than 8 AD cases. Also, the teraromic isomer of AChE is a marker of cholinergic innervation) was not reduced. Our results suggest that pathological alterations of the basal forebrain cholinergic system do not precede SF development. CHAT activity and AChE isomorph patterns in hippocampus are unaffected by severe loss of CA1 pyramidal neurons.


The primary cause of Alzheimer disease (AD) remains unknown, among the disease pathogenic factors that have been suggested, based on autосomal dominant transmission of the disease in several pedigrees. Neurofibrillary tangles (NFTs), paired helical filaments (PHFs), and neurofibrillary inclusions (NFTs) are the critical neuropathological feature for the differential diagnosis of Alzheimer's disease (AD). Immunological and antigens-specific antibodies demonstrate cross-reactivity with "nitrified" PHF. It has not been shown, however, that the PHF cross-reactivity is associated with the insoluble PHF core protein (PHF) as opposed to PHF associated proteins or components of the purification scheme. We determined the aminoacids sequence of PHFs (105 colonies) from 1.E4 virus-like particles in Tritor/Bof/SDS buffer based on the separation of palmated PHF associated proteins (PHF) from PHF. Electromicroscopy revealed an intact PHF structure before and after this ultrafiltration technique (UF). A significant difference in aminoacids sequence of the PHF core protein was demonstrated. The percent total mass of hydroxyproline (Hyp) and glycine increased in the PHF after UF. The elevation of these amino acids in PHF remained constant from 1.5 to 18 hours after UF, peaking at 2% of the total protein after 39 hours. ELISA data confirmed that our PHF and PHF fractions were reactive with several lesions PHF-specific antibodies (Abs). Cross-reactivity of PHFs with Abs to other cellular components which includes: a broad range of post-mortem delay, and a newly identified determination, was demonstrated. None of the purported components of PHF contain Hyp. Therefore, these data suggest that inappropriate hydroxylation of proline residues in PHF proteins occur in AD. This hydroxylation may be the catalyst for the polymerization and subsequent insolubility of PHF in brain regions affected with AD.

437.24 DISCOVERY OF HYDROXYPROLINE AND AN ACTIV DETERMINANT IN THE ISOLABLE PARTIAL HELICENTRIC FIBRILS OF ALZHEIMER BRAIN: M. A. Tomiyama, R. J. Snyder and P. P. Zeman. Department of Physiology and Biophysics, Department of Neurobiology and Molecular Genetics, and The Alzheimer's Research Center, University of Cincinnati College of Medicine 45267.

Neurofibrillary tangles (NFTs), comprised of paired helical filaments (PHFs), are critical neuropathological feature for the differential diagnosis of Alzheimer's disease (AD). Immunological and antigens-specific antibodies demonstrate cross-reactivity with "nitrified" PHF. It has not been shown, however, that the PHF cross-reactivity is associated with the insoluble PHF core protein (PHF) as opposed to PHF associated proteins or components of the purification scheme. We determined the aminoacids sequence of PHFs (105 colonies) from 1.E4 virus-like particles in Tritor/Bof/SDS buffer based on the separation of palmated PHF associated proteins (PHF) from PHF. Electromicroscopy revealed an intact PHF structure before and after this ultrafiltration technique (UF). A significant difference in aminoacids sequence of the PHF core protein was demonstrated. The percent total mass of hydroxyproline (Hyp) and glycine increased in the PHF after UF. The elevation of these amino acids in PHF remained constant from 1.5 to 18 hours after UF, peaking at 2% of the total protein after 39 hours. ELISA data confirmed that our PHF and PHF fractions were reactive with several lesions PHF-specific antibodies (Abs). Cross-reactivity of PHFs with Abs to other cellular components which includes: a broad range of post-mortem delay, and a newly identified determination, was demonstrated. The percent total mass of hydroxyproline (Hyp) and glycine increased in the PHF after UF. The elevation of these amino acids in PHF remained constant from 1.5 to 18 hours after UF, peaking at 2% of the total protein after 39 hours. ELISA data confirmed that our PHF and PHF fractions were reactive with several lesions PHF-specific antibodies (Abs). Cross-reactivity of PHFs with Abs to other cellular components which includes: a broad range of post-mortem delay, and a newly identified determination, was demonstrated. None of the purported components of PHF contain Hyp. Therefore, these data suggest that inappropriate hydroxylation of proline residues in PHF proteins occur in AD. This hydroxylation may be the catalyst for the polymerization and subsequent insolubility of PHF in brain regions affected with AD.


Using SDS polyacrylamide gel electrophoresis and Western blotting we have compared GAP-43 from rat and human cortex, and examined levels of GAP-43 in normal and Alzheimer's disease (AD) hippocampus. GAP-43 was identified in western blots using a mouse monoclonal antibody (H9124) directed against rat GAP-43 (antibody provided by B.H.P. Skene, Stanford University) followed by biotinylated horse antimouse IgG, avidin-biotin complex, and peroxidase substrate.

GAP-43 was detected both in the membrane-bound and cytosolic fractions of cell extract. Triton X-100 extraction of the membrane were able to remove some GAP-43, but complete extraction required SDS in the membrane fractions. We find that the rat and human GAP-43 show the same molecular weight. In order to determine if post-mortem delay leads to changes in GAP-43, we have examined rat brain subjected to a variety of post-mortem conditions. Our data suggest that a broad range of post-mortem delay has little effect on amount or electrophoretic pattern of GAP-43.

In paired of AD and control cases matched for age and post-mortem delay, we find lower levels of GAP-43 in SDS extracts from AD hippocampi.

When GAP-43 was analyzed from SDS gels containing neuraminidase, we observed an increase in the heterogeneity of the protein. In these gels the GAP-43 from human tissues appears to be more heterogeneous than the rat GAP-43.

Supported by grants AG 1121, AG 107, AG 03644, and ADRA 87-053.


We have used an in vitro system to investigate the response of the nervous tissue to stress conditions. Explants of rat dorsal root ganglia were exposed to two different types of stress conditions: aluminum intoxication and heat shock. Expression and cellular location of Heat Shock Proteins (HSPs) were analyzed by immunofluorescence and immunoblotting after separation by one and two dimensional gel electrophoresis, and by immunocytochemistry using mono and polyclonal antibodies. Ubiquitin (Ub) conjugates were also analyzed. Both stress conditions resulted in an increase of the Ub-conjugates, with an higher increment in chronic aluminum intoxication. On the contrary, HSPs were detected only after heat shock treatment. These findings confirm the hypothesis that the heat shock system can be triggered independently by different types of stress conditions: aluminum intoxication may be relevant to the recent discovery that Ub is present in neuronal inclusions of Alzheimer's and other degenerative diseases, whereas, on the contrary, HSPs have not been detected. (Supported by NIH Grants NS14503 and AG00793).
**ION CHANNELS: MODULATION AND REGULATION**

**438.1** EFFECTS OF CARBACHOL AND GALLAMINE ON WHOLE-CELL CURRENTS IN NEURONS IN Slices OF NEONATAL R. S. Doerner and B. E. Alger, Dept. of Physiol., Univ. of Maryland Sch. Med., Baltimore, MD 21201.

The actions of carbachol (CCh) and gallamine were studied in mouse neuroblastoma-glioma N1E-115 cells under whole-cell voltage-clamp. Using K-filled electrodes, brief pressure-ejection of CCh (0.05-1 mM) from blunt pipettes elicited a transient inward current followed by a longer-lasting outward current. Gallamine (100-200 µM), a nicotinic and putative muscarinic M2 receptor antagonist, had no effect on resting membrane current but blocked the CCh-induced inward current without altering the outward phase. Aromatic reduced the outward component, but did not affect the inward transient.

Both application of CCh reversibly suppressed voltage-dependent K current and decreased membrane conductance. Using Ca-filled electrodes and saline containing Ba and TTX, CCh application reversibly reduced peak inward I\(_{\text{Ba}}\) elicited by depolarizing clamp steps from hyperpolarized holding potentials. This effect was inhibited by atropine but unaffected following bath application of gallamine. Characteristics of the I\(_{\text{Ba}}\) 1/A relationship were unaffected by CCh.

These data suggest that nicotinic receptor activation initiates an inward current while muscarinic responses involve both activation of an outward current and modulation of voltage-gated channels.

**438.2** TWO MEMBRANE CONDUCTANCE RESPONSES OF DISSOCIATED RAT SYMPATHETIC NEURONS TO MUSCARINE ARE COUPLED BY A PTX-SENSITIVE GTP-BINDING PROTEIN. N.V. Marrion*, T.G. Smart* and D.A. Brown, MRC Neuropharmacology Research Group, Departments of Pharmacology, London University School of Pharmacy, London, WC1N1AX, U.K.

Dissociated adult rat superior cervical sympathetic neurons (Marrion, N.V., Smart, T.G. & Brown, D.A., Neurosci. Lett. 26:209, 1981) in a proportion of cells an additional component of inward current due to an increase in a time- and voltage-sensitive conductance, insensitive to TTX, CCh application reversibly reduced peak inward I\(_{\text{Ba}}\) elicited by depolarizing clamp steps from hyperpolarized holding potentials. The membrane current was reversed near the membrane potential and to voltage-clamp neurons in the single electrode configuration, using whole-cell suction electrodes. The patch pipette solution contained: (in mM) KCl, 140; NaCl, 5; CaCl\(_2\), 2; MgCl\(_2\), 1; EGTA, 0.5; HEPES, 5; NaOH, 3. (pH 6.7). This solution preserves the voltage-clamp characteristics of the patch pipette, and to maintain the voltage-clamped condition, the patch pipette contained 200-400 mV of the membrane potential.

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The role of the second messenger diacylglycerol [DAG] in mediating muscarinic suppression of M-current, a type of K current in smooth muscle cells isolated from Bufo marinus was examined using single microelectrode voltage-clamp techniques. Extracellular application of 100-1,000 µM 1,2-diacylglycerol (1,2-DAG), a synthetic DAG analog, reversibly suppressed endogenous M-current in the same way as acetylcholine [ACh], causing a net current associated with a conductance decrease. Current relaxations which occur in response to hyperpolarizing pulses and represent the voltage-dependent turn-off of M-current were also decreased by 1,2-DAG. The suppression of M-current by 1,2-DAG was not always as complete as it was in the case of ACh. Like ACh, 1,2-DAG also suppressed M-currents induced by isoproterenol through the mediation of cyclic AMP (Sims et al. Science 239: 190, 1988). Thus, the dual regulation of M-current by isoproterenol and ACh appears to involve two second messenger systems. Supported by NIMH DK31620 and NSF DCB5811674.

**438.4** MUSCARINIC RECEPTORS MAY BE UNCOUPLED FROM INWARDLY RECTIFYING POTASSIUM CURRENT IN DISSOCIATED CELL CULTURE. L. E. Frischini. Dept. of Neurology, Emory Univ. School of Med., Atlanta, GA 30322.

We have found that neurons dissociated from neonatal rat superior cervical ganglia frequently lack electrophysiological responsiveness to muscarinic agonists. We have undertaken studies to determine how these neurons lose their responsiveness and how expression may be reestablished. We grew neurons in 12 medium coverslips with 105 each from 1-day old Sprague-Dawley rat pups (60 µM norepinephrine) to maximize growth factor release (50 µM norepinephrine). We co-cultured some neurons with previously plated confluent brain glia, and we treated some cultures with conditioned medium from rat heart cell lines in culture. We used the cell ball technique to measure membrane potential and to stimulate neurons with single or paired pulses of a voltage-clamped clamp mode (Aclamp-Zs). Neurons that were unresponsive to muscarine (1 µM to 1 mM) expressed outward rectifying potassium current with a conductance of 10-20 mS/cm², while those that were responsive to muscarine showed a net inward current as associated with a conductance as large as several nA which reversed near the membrane potential and to voltage-clamp neurons in the single electrode configuration, using whole-cell suction electrodes. The patch pipette solution contained: (in mM) KCl, 140; NaCl, 5; CaCl\(_2\), 2; MgCl\(_2\), 1; EGTA, 0.5; HEPES, 5; NaOH, 3. (pH 6.7). This solution preserves the voltage-clamp characteristics of the patch pipette, and to maintain the voltage-clamped condition, the patch pipette solution contained 200-400 mV of the membrane potential.

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SEROTONIN DIFFERENTIALLY MODULATES TWO K+ CURRENTS IN THE LECHseite Rigel (R) cell of the Leech, Ha. E.C. Urdahl, Dept. of Pharmacology, University of Wisconsin, Madison, Wis., 53706.

Introduction

We have used a patch clamp technique to study the effects of various drugs on the potassium currents in the leech Rcell. We have found that potassium currents are modulated by a variety of drugs, including serotonin (5-HT) and bradykinin (BK).

Materials and Methods

We used the patch clamp technique to record potassium currents in the leech Rcell. The cells were held at a potential of -60 mV and the currents were recorded with a minimal amount of extracellular solution.

Results

1. Serotonin (5-HT) increases potassium currents in the leech Rcell. This effect is dose-dependent and is blocked by the 5-HT receptor antagonist, 8-OH-DPAT.

2. Bradykinin (BK) decreases potassium currents in the leech Rcell. This effect is dose-dependent and is blocked by the BK receptor antagonist, HOE 140.

3. The effects of serotonin and Bradykinin on potassium currents are not additive. The combination of 5-HT and BK results in a net increase in potassium currents.

Discussion

These results suggest that potassium currents in the leech Rcell are modulated by 5-HT and BK, and that the effects of these drugs are not additive. The combination of 5-HT and BK results in a net increase in potassium currents, suggesting that these drugs may act through a common mechanism.

References


Calmodulin-dependent protein kinase (CaM kinase II) has been shown to modulate specific K+ and Ca2+ currents in vertebrate neurons. To investigate a possible role of this kinase in vertebrate neurons, we have intracellularly injected purified CaM kinase II into spinal cord (SC) neurons cultured from embryonic rats. Each spike in a cultured SC neuron is followed by a large (5-10 mV) and prolonged (30-50 ms) after-hyperpolarization (AHP). Injection of CaM kinase II resulted in a reduction in both amplitude and duration of AHP, and sometimes spike broadening (n=11 cells). CaM kinase II effects on AHP were observed 4 min post-injection and peaked after 5-6 min. In some cases, AHP was totally abolihed or not heat-inactivated CaM kinase II injection (n=8 cells) produced these effects. Preliminary studies suggest AHP is enhanced by injection of CaM kinase II monoclonal antibody that inhibits CaM kinase II activity in vitro and labels SC neurons immunochemically. This observation suggests a role for endogenous CaM kinase II in modulating AHP. Characterization of AHP indicates that it is due predominantly to Ca2+-dependent K+ conductances (Zhang & Krnjevic, Neurosci. Lett. 74:68, 1986). These results indicate that CaM kinase II regulates AHP in SC neurons and are consistent with the hypothesis that the effects of CaM kinase II on K+ and/or Ca2+ currents may be responsible for the effects of this kinase on AHP.

438.15 ACTION POTENTIAL CHARACTERISTICS OF SEGMENTALLY DEMYELINATED OPTIC TRACT (OT) AXONS FOLLOWING POTASSIUM CHANNEL BLOCKERS. D. A. Fox, D-Y. Yuan and Y. Blocker*. U. of Houston, College of Optometry, Houston, TX 77004.

The role of potassium current blocking agents in altering conduction properties of demyelinated rat optic tract axons has been examined, however, similar studies have not been conducted in CNS axons. Our in vivo studies examined the frequency-dependent effects of voltage (V), 4-AP and 4-AP + TEA on compound action potential (CAP) characteristics and excitability properties in fast (ci) and middle conducting (ct) OT axons in two different models of demyelination, segmental and paranodal (see following abstract), after optic chiasma stimulation. Segmental demyelination, produced by developmental and continuous lead exposure, was confirmed in 90 day old hooded rats using electron microscopy. In t2 and t1, lead decreases conduction velocity and amplitude (AMP), and increases rise (RT) and fall time, duration (DUR), and chronaxie. In t2, lead increases absolute and relative refractory periods (RFP), decreases frequency following (FF) and produces a subnormality. Compared to baseline, 4-AP effects are generally larger in t2 than in t1 (e.g., 4-AP decreases AMP and RF, and increases DUR, RT, and RFP). TEA effects are similar to those produced by 4-AP, however, as stimulus frequency increases they are larger in t1 than in t2. Large and medium diameter axons exhibit differential frequency-dependent sensitivity to 4-AP and TEA following segmental demyelination. Supported by ES 03183 (DAF).

438.16 ACTION POTENTIAL CHARACTERISTICS OF PARANODALLY DEMYELINATED OPTIC TRACT (OT) AXONS FOLLOWING POTASSIUM CHANNEL BLOCKERS. D-Y. Yuan, D. A. Fox and Y. Blocker*. U. of Houston, College of Optometry, Houston, TX 77004.

The role of potassium channel blocking agents in altering conduction properties of demyelinated rat optic tract axons has been examined, however, similar studies have not been conducted in CNS axons. Our in vivo studies examined the frequency-dependent effects of voltage (V), 4-AP and 4-AP + TEA on compound action potential (CAP) characteristics and excitability properties in fast (ci) and middle conducting (ct) OT axons in two different models of demyelination, segmental and paranodal (see following abstract), after optic chiasma stimulation. Paranodal demyelination, produced by 2,3-benzenedicarboxylic acid (2,3-BDA), was confirmed in 7-month old hooded rats using electron microscopy. 2,3-BDA decreases conduction velocity, amplitude, rheobase and frequency following and increases rise and fall time, duration, chronaxie and absolute refractory period in t1 and t2. t2 axons have hyperpolarization and a correlated decrease in relative refractory period. The CAPs were almost identical to those observed in lead-exposed rats with segmental demyelination (see preceding abstract); 4-AP effects are generally larger in t2 than in t1. In contrast, TEA effects are only observed at low stimulus frequencies and generally only in t2. Paranodally and segmentally demyelinated large and medium diameter OT axons have similar sensitivity to 4-AP and completely different sensitivity to TEA. Supported by ES 03183 (DAF).


The CaM-activated Na+ current (INa,cAMP) in molluscan neurons is independent of phosphorylation and diffusion-limited in its kinetics. These characteristics permit precise calculation of CaM diffusion, INa,cAMP, and phosphodiesterase (PDE) kinetic parameters.

Use of a diffusion-limited model permits precise calculation of the Na+ current INa,cAMP by pulsed intracellular injection of CaM, including effects of relocating the point source of CaM within the cell, and effects of PDE inhibitors. The apparent diffusion coefficient of CaM is estimated from the relation of latency to peak Na+ current and cell radius. PDE activity is extracted as a first-order rate constant from the exponential decay of INa,cAMP, while Michaelis-Menten parameters (Km, Vmax) were obtained by curve-fitting the INa,cAMP response to an explicit finite difference equation.

Data indicate one-to-one binding of the ligand. A competitive binding mechanism explains apparent voltage-dependence of CaM dissociation. The IV curve for INa,cAMP, and antagonists of CaM suppression effects by high levels of intracellular CaM and in regulating channel activity. Block Ca2+ suppresses, while CaM stimulates, ion current. Binding of one ligand antagonizes binding of the other. Experimental and theoretical predictions agree well.
PRESYNAPTIC MECHANISMS I

THURSDAY PM

DIFFERENT Ca++ SENSITIVITY OF SPONTANEOUS RELEASE ALONG THE FROG NEUROMUSCULAR JUNCTION. J.P. Tremblay and R.P. Robitaille, Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4.

349.3 DIFFERENT CA++ SENSITIVITY OF SPONTANEOUS RELEASE ALONG THE FROG NEUROMUSCULAR JUNCTION. J.P. Tremblay and R.P. Robitaille, Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4.

349.4 NONUNIFORM TRANSMITTER RELEASE EFFICACY ALONG THE FROG NEUROMUSCULAR JUNCTION. J.P. Tremblay, Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4.


349.6 INHIBITORS OF VESICULAR ACETYLCHOLINE TRANSPORT AT MOUSE AND FROG NEUROMUSCULAR JUNCTIONS. J.P. Yut and W. Van der Knaap, Dept. of Physiology & Biophysics, SUNY, Stony Brook, NY 11794.

PRETREATMENT IN HYPERTONIC SOLUTION INCREASES MEPPS AND UN-QUANTAL EPS SIZES ROUGHLY TWOFOLD. Responses to iontophoretic or bath applied Ach were unchanged by the pretreatment. In frog nerve-muscle preparations the inhibitors had little effect on the release of Ach, but in rat preparations the inhibitors promptly decreased the size of mouse MEPPs and MEPCs about 50%.

The present experiments were undertaken to confirm our previous observation that the MEPP amplitudes were smaller in the distal region of the frog neuromuscular junction (NMJ) than in the proximal region (near point of contact between the axon and the muscle fiber). These results were obtained with the Spatial Delay Method using simultaneous intracellular recording with 2 electrodes (Tremblay et al. Soc. Neurosci. Abstr., Vol. 15 Part 1, p. 371) and/or smaller active zones in the distal regions. To further investigate this finding, intracellular recordings were made in presence of 6-8mM neostigmine with an electrode placed halfway between the distal end and the proximal region. During the intracellular recording period an extracellular electrode was placed alternatively at a distal and at a proximal site. The temporal correspondence between the extracellular and intracellular signals permitted us to identify two MEPP populations originating from the distal and proximal regions. In the majority of these experiments we observed that the intracellularly recorded MEPPs originating from the distal region were significantly smaller than those produced in the proximal region of the same NMJ. This confirms Tremblay et al. observations using the Spatial Delay Method. A significant correlation was observed between the amplitude (in pA) and the area (in µV ms) of the MEPPs recorded at the distal sites. The results suggest that Ach receptors in the distal region are characterized by shorter PPFs. Thus our observations suggest that Ach would reduce fewer times to Ach receptors in the distal region characterized by shorter PPFs.
PRESYNAPTIC MECHANISMS I

There are two major classes of neurotransmitter: excitatory and inhibitory. Excitatory neurotransmitters, such as glutamate and acetylcholine, increase the probability of an action potential being generated at the postsynaptic neuron. Inhibitory neurotransmitters, such as GABA (gamma-aminobutyric acid) and glycine, decrease the probability of an action potential being generated.

GABA is released from the presynaptic terminal and binds to receptor proteins in the postsynaptic membrane, which results in an inward current and a decrease in the probability of an action potential being generated. This is known as a postsynaptic inhibitory potential (IPSP).

The process of neurotransmitter release involves the following steps:

1. **Depolarization**: The release site is depolarized by the arrival of an action potential, which opens voltage-gated calcium channels and allows calcium ions to enter the presynaptic terminal.

2. **Calcium Influx**: Calcium ions enter the presynaptic terminal and bind to calcium/calmodulin-dependent protein kinase II (CaM K II), which results in the phosphorylation of specific residues.

3. **Phosphorylation**: The phosphorylated residues allow vesicles to move to the active zone and fuse with the presynaptic membrane, releasing neurotransmitter into the synaptic cleft.

4. **Release**: Neurotransmitter is released into the synaptic cleft and diffuses to the postsynaptic membrane.

5. **Receptor Activation**: The neurotransmitter binds to its specific receptor, which activates an ion channel or a second messenger system.

6. **Second Messenger System**: In the case of GABA, the receptor activation results in an inward current, which reduces the probability of an action potential being generated.

7. **Termination**: Neurotransmitter is removed from the synaptic cleft by diffusion, reuptake, or enzymatic degradation.

The process of neurotransmitter release is highly regulated and involves many different proteins. The structure and function of these proteins are critical for the proper functioning of the nervous system.

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**References**


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**Note**

The above text is a simplified explanation of the process of neurotransmitter release. For a more detailed explanation, please refer to the primary文献。

The Ca2+-dependent release of dopamine (DA) and acetylcholine (ACH) from the striatum is modulated by DA D2 receptors. However, the mechanism of this release inhibition is unknown. We have studied 3H-DA and 14C-ACH release from superfused rabbit striatal slices in the presence of phorbol-12,13-dibutyrate (PDBu) and forskolin (FSK). We determined if protein kinase C (PKC) and cAMP phosphorylate or inhibit stimulated 3H-DA and 14C-ACH release. FSK also enhanced stimulated, but not basal release of 3H-DA (EC50=100nM). 14C-ACH release was unaffected. In the presence of PDBu, DA D2 receptor agonists were less potent and less effective at inhibiting 3H-DA and 14C-ACH release. Also, the DA D2 receptor antagonist sulpiride was less effective at enhancing stimulated 3H-DA and 14C-ACH release. FSK also enhanced stimulated, but not basal release of 3H-DA (EC50=100nM). 14C-ACH release was unaffected. FSK did not alter the inhibition of 3H-DA and 14C-ACH release produced by D2 agonists.

These results suggest that a) activation of adenylate cyclase and FSK may facilitate DA release and b) D2-receptor modulation of DA and ACh release may be regulated by FSK function.

**439.15**
IN SITU PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN RAT STRIATAL SYMPATHETIC NEURONS, BOVINE ADRENAL CHROMAFFIN CELLS, AND PC12 CELLS: DIFFERENCES IN PHOSPHOPROTEINS. J.W. Hayrock, M. Calab* and D. Morgan*. Dept. Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119.

Incubation of catecholaminergic tissues with [32P]Pi leads to the incorporation of [32P]Pi into tyrosine hydroxylase (TH), the rate-limiting and initial step in catecholamine biosynthesis. Depolarization produces a calcium-dependent increase in the phosphorylation of TH independent of [32P]Pi; and, a sustained increase in TH phosphorylation may be involved in modulating this effect.

In the present studies we compared the HPLC elution profiles of tryptic TH phosphopeptides from synaptosomes, solubilized rat and bovine tissue, prior to immunoprecipitation, failed to reveal any identity of any of the bovine and rat TH phosphopeptides. The amino acid sequences of some of the tryptic peptides, as inferred from previously published [CDNA sequences, differ at only one amino acid (gln vs leu) in vivo. The possibility that either genetic differences or tissue-specific, post-translational modifications may account for the observed differences in retention times is currently being investigated.

**439.16**

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, has been shown to be phosphorylated in a variety of preparations both in vivo and in situ. One essential criterion for determining the physiological significance of such findings is whether TH is phosphorylated in vivo. By infusing [32P]Pi (2 mCi, 5 u, 60 min) and rapidly fixing the striata by liquid nitrogen injection (Neuman. Abstr. (1986) 12:737), we have measured [32P] incorporation into TH in vivo. [32P]-TH, after immunoprecipitation and SDS-PAGE, showed no evidence of proteolysis. The TH bands were subjected to limited tryptic digestion, and the resulting phosphopeptides were separated by HPLC and analyzed with on-line radiochemical detection. The elution profile of these phosphopeptides was similar to that previously described for TH that was labeled in situ in the striatum of rats (Brain Res. Bull. (1987) 19:519). Electrical stimulation (biphasic square wave, 200 uA, 3 ms duration, 30 Hz, 20 min) of the medial forebrain bundle produced a change in the ratio of [32P] incorporation into two of the four major phosphopeptides. This change in the pattern of [32P] incorporation into TH phosphopeptides was different from that previously reported for TH in situ. Synaptosomes and PC12 cells: differences in TH phosphorylation by electrical stimulation of the nigro-striatal pathway in vivo may differ from that produced in situ.

DA neurons in the neostriatum (NS) possess inhibitory pre-synaptic receptors (alpha-2 heteroreceptors) stimulating another neurotransmitter. Clonidine has been shown to inhibit potassium (K+) stimulated 3H-DA release from rat nucleus accumbens (NA) slices, suggesting the presence of alpha-2 heteroreceptors in that region as well. We assessed the effects of the alpha-2 agonist d-medetomidine on K+ stimulated 3H-DA release from rat NA and NS synapses in superfusion system. In NA, control release of the total was 18.6±1.1% (mean ±SEM) of 3H-DA initially present in the synaptosomes. Unlabeled DA (1.8µM) reduced release to 13.1±1.4% (p<.001 vs. control) consistent with an action on autoreceptors. Similarly, d-medetomidine (10µM) reduced 3H-DA release to 13.6±1.0% (p<1.102, p=.001 vs. control). d-Medetomidine (1.0µM) had little effect on 3H-DA release in the NS, with a mean ±SEM of 25.1±0.8%, not significantly different from the control value. Our data suggest that alpha-2 heteroreceptors are present on only a subset of central DA terminals and imply that it may be possible to selectively modify central DA activity with alpha-2 agonists and antagonists.


Two independent presynaptic mechanisms, one involving modulation of ionic currents and the other an increase in the availability of transmitter for release, were characterized in heterogeneous synaptic facilitation at the sensorimotor synapses of Aplysia. To study the second of these mechanisms, we observed the spontaneous miniature epsps at synapses formed between single sensory and motor cells in vitro. 1-10 uM 5-HT reversibly increased the frequency of spontaneous release 4-5 times while the inhibitory peptide FMRFamide (100 nM) reversibly reduced the frequency of spontaneous release to near control values when applied simultaneously with the 5-HT. A major component of the modulation of spontaneous release does not depend on either a Cax influx or changes in the concentration of internal Ca. In salines lacking Ca or containing 1 mM Cd to block Ca currents, 5-HT still enhanced the rate of release by 3-4 times, and FMRFamide when applied simultaneously reduced the rate to near control values. When BAPTA was injected into the sensory neuron to buffer the internal Ca concentration, 5-HT and FMRFamide still modulate spontaneous release. The rate of spontaneous release, and consequently the transmitter available for evoked release during presynaptic inhibition and presynaptic facilitation, can therefore be modulated by 5-HT and FMRFamide directly by a mechanism which is independent of changes in internal Ca.


Nicotine stimulates the release of [3H]GABA and [3H]ACh from hippocampal synaptosomes. The presynaptic nicotinic acetylcholine receptor (nAChR) mediating this action has a pharmacological specificity similar to that of the high affinity binding sites for [3H]nicotine in brain membrane fractions. (nAChRs) 230 and 290 which are specific for these nAChRs, we have immunoisolation isolated synaptosomes bearing surface ['3H]nicotinic acetylcholine receptors. Synaptosomes, highly purified from rat hippocampus by isotonic Percoll density gradient centrifugation, were enriched in ['3H]nicotinic binding sites and high affinity transport of ['3H]GABA and ['3H]choline. Synaptosomes were incubated with tubes 270 and 290 coupled to Dynabeads M-450 (Dynal) and bound synaptosomes were separated magnetically. Up to 25% of the total nerve terminal population could be immunoisolated, as judged by measurement of lactate dehydrogenase. A differential purification of choline and GABAergic terminals, assessed by CAT and GAD activities, respectively, was observed. These results suggest that nAChR are present on 25% of rat hippocampal nerve terminals, including choline and GABAergic terminals consistent with the presynaptic modulation of transmitter release by these nAChRs. (Supported by R.J.Reynolds Co.)


There is general agreement that the enhancement of neurotransmitter release from motor nerve terminals by norepinephrine is mediated by α1-adrenoceptors (Chen and Dryden, Neurosci. Lett. 13:18, 1987). However, our recent studies in mouse hippocampal slices (Chen and Dryden, Neurosci. Lett. 68:3, 1987) suggested that the response involved a cyclic AMP link and this conflict with the existing concepts of transduction at the α1-adrenoceptor. The present study was undertaken to identify the mechanism involved at this site. NEP frequency was increased at 20°C in mouse hippocampal slices exposed to 150mM [K+]o. The enhanced release of quanta caused by 1µM NE was reduced by non-specific cyclic nucleotide inhibitors (30µM theophylline, 1.2 µM polymyxin B, 20µM aurofuran) but was not affected by 50 µM H3, an inhibitor of cyclic nucleotide dependent kinase and protein kinase C. It was reduced by 100µM H3, an inhibitor of calmodulin-dependent (CaM) kinases. The effect of NE was increased by 10µM L1, which prevents further metabolism of inositol triphosphate (IP3) but was unaffected by pertussis toxin (2.5 µg/ml for 4 hours at 37°C). These results are consistent with transduction by IP3, as Ca2+ release and activation of a CaM kinase. (Supported by Univ. of Alberta Central Research Fund).

440.5 NORADRENERGIC (NA) MEDIATES A COMPONENT OF VIP RELEASE EVOKED BY 4-AMINOPYRIDINE (4-AP) IN MOUSE NEOCORTEX. L.J. Martin and P.J. Magistretti. Departement de Pharmacologie, CMU, 1211 Geneva 4, Switzerland.

In mouse cerebral cortical slices, 4-AP stimulates in a concentration-dependent manner basal VIP release (VR). VR evoked by 4-AP 1 mM is completely blocked by Co2+ 2.5 mM and partially inhibited by TTX 2 µM. Moreover, an inhibitor of phosphatase A, inhibits 4-AP-elicited VIP release in 10 mM of EGTA, 15 µM of indomethacin, a cyclooxygenase inhibitor, and 15 µM of nitrobenzyl tetrahydroindole (NBTHI) 2 ms, an inhibitor of sodium-Na/K-ATPase, blocked accommodation to depolarizing current pulses, and decreased, suggesting its effect is presynaptic. PILO reduced EPSPs when applied simultaneously, but was not affected by 100 µM TTX, an inhibitor of cyclic nucleotide dependent kinase and protein kinase C. It was reduced by 100 µM TTX, an inhibitor of calmodulin-dependent (CaM) kinases. The effect of NE was increased by 10µM L1, which prevents further metabolism of inositol triphosphate (IP3) but was unaffected by pertussis toxin (2.5 µg/ml for 4 hours at 37°C). These results are consistent with transduction by IP3, as Ca2+ release and activation of a CaM kinase. (Supported by Univ. of Alberta Central Research Fund).


Pilocarpine (PILO)-induced status epilepticus (SE) in mice is potentiated by lithium. This interaction was investigated in hippocampal slices. Slices were taken from 150-350 gm rats, incubated in an oxygenated solution and stimulated with 4 hours at 37°C. Two 15-45 min trains of 100 µsec pulses were delivered. PILO had effects like other muscarinic agonists: it increased input resistance, blocked a slow AHP, blocked accommodation to current pulses, and had no effect on the excitability of the hippocampus. These inhibitory effects of arachidonic acid metabolites' formation act exclusively on the TTX-sensitive component of 4-AP. NA potentiates in a concentration-dependent manner basal VIP release (VR). VR evoked by 4-AP 1 mM is completely blocked by Co2+ 2.5 mM and partially inhibited by TTX 2 µM. Moreover, an inhibitor of phosphatase A, inhibits 4-AP-elicited VR release in 10 mM of EGTA, 15 µM of indomethacin, a cyclooxygenase inhibitor, and 15 µM of nitrobenzyl tetrahydroindole (NBTHI) 2 ms, an inhibitor of sodium-Na/K-ATPase, blocked accommodation to depolarizing current pulses, and decreased, suggesting its effect is presynaptic. PILO reduced EPSPs when applied simultaneously, but was not affected by 100 µM TTX, an inhibitor of cyclic nucleotide dependent kinase and protein kinase C. It was reduced by 100 µM TTX, an inhibitor of calmodulin-dependent (CaM) kinases. The effect of NE was increased by 10µM L1, which prevents further metabolism of inositol triphosphate (IP3) but was unaffected by pertussis toxin (2.5 µg/ml for 4 hours at 37°C). These results are consistent with transduction by IP3, as Ca2+ release and activation of a CaM kinase. (Supported by Univ. of Alberta Central Research Fund).
M.L. Csizy*, B.J. Frost. Queen's University, Kingston, with great accuracy. The auditory localization of nocturnal raptor which acoustically locates its prey playback sounds of mice moving through leaves. Saw-whet owl (Aegolius acadicus) is a pronounced asymmetry in the bone structure of the ear openings, and has extremely acute sound localization ability (Csizy and Frost, 1988). The owl's interaural axis may be a function of the biological irrelevance of the sound source location. Cells in lateral MLD were sharply tuned to frequencies from 200 Hz, represented dorsally, through 7 kHz, overlaid MLD. Cells responded preferentially to wide-band noise stimuli, and showed spatial selectivity in both azimuth and elevation. Supported by NSERC Grant A0353 to B.J.F.

Sensory Systems: Auditory Systems VII

440.7 INTERACTIONS OF MŁMA STEROISOMERS ON HIGH AFFINITY UPTAKE IN RAT HIPPOCAMPAL SYNAPTOSOMES. Poblete, J. Cannon, L.T. Byrnes, B.J. Frost, J. Neary, T. and Dept. Psychiatry, SUNY, Stony Brook, NY 11794. We investigated the interactions of the (R) and (S) enantiomers of MŁMA on high affinity uptake of [3H]-5-HT in synaptosomes from hippocampal tissue. Enantiomers were used at concentrations (around 10^-4M) and inhibited at high concentrations (around 10^-2M). The [3H]-5-HT uptake on cultured serotonergic neurons. The (R)-MŁMA was inhomogeneous in the same ranges. Multiple applications of MDMA enhanced the enantiomers produced increases in the number of [3H]-5-HT neurons grown in normal or Nb Ca^2+ media. The S-MŁMA enantiomers were synergistically enhanced survivors of 5-HT neurons, their somal area and process length. In lowest, the (S) enantiomer produced a decrease in somal area and process length of 5-HT neuron. The specific L-channel Ca^2+ antagonist, nimodipine (2x10^-5M), blocked the enhancement of L-glutamate (Glu) and dynorphin from a subcellular fraction of hippocampal MF synaptosomes. Endogenous Glu and dynorphin A(1-8) release from normal or no Ca^2+ media, the uptake of [3H]-5-HT was 50% lower after 30 min. Under these conditions, the (R) isomer exhibited a more profound inhibition of L-glutamate and dynorphin release, whereas the (S) isomer had a lower effect on the same neurotransmitter. The long-term potentiation (LTP) of these data suggest that one high affinity binding site exists for both enantiomers of MŁMA. The (R) isomer has a more potent effect of 5HT uptake in rat hippocampal Synaptosomes. Supported by NIDA contract 271-87-8144.

440.8 PREVENTION OF MŁMA TOXICITY TO SEROTONERGIC NEURONS IN NRCYCLIC CULTURE BY ADENOSINE AND DYNORPHIN. Azmitia, E.C.* Murphy, B.L., Whitaker-Azmitia, E.M. School of Aerospace Medicine, Brooks AFB, TX 78235-5301. The Electrophysiological studies indicate that the anti-convulsant effect of adenosine and its analogues on hippocampal CA3 pyramidal neurons is primarily due to a pre-synaptic inhibition of excitatory neurotransmitter release. The hippocampal mossy fibre (MF) system provides a major excitatory synaptic input to the CA3 subfield and may represent a site of adenosine action. We reported that the specific L-channel Ca^2+ antagonist, 2-chloroadenosine (ClAdo) preferentially inhibits the Ca^2+-dependent component of glutamate release from cultured MF synaptosomes. In the present experiments, we have investigated the mechanism by which adenosine modulates the MF synapse. The results demonstrate that ClAdo (0.1 µM) inhibits the 45 µM K^+-evoked release of dynorphin A(1-8), as well as glutamic acid, from superfused MF synaptosomes. Exogenous ATP (0.1 - 1.0 mM) did not enhance the effect. The order of agonist potency is CA3 > cytochexyladenosine > 5'-N-ethylcarboxamidoadenosine. The IC50 for the effects of ClAdo on glutamic acid release is near 0.1 µM and this inhibition can be prevented by increasing the concentration of calcium from 0.5 to 7.0 mM. These findings suggest that adenosine presynaptically modulates the hippocampal MF synapse by interfering with calcium influx or its availability within the nerve terminal.

440.9 MODULATION OF GLUTAMATE AND DYNORPHIN RELEASE FROM HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. P.G. Hernandez*, R.L. Cannon, M.A. Rea and D.M. Terrain. U.S.A.F. School of Aerospace Medicine, Brooks AFB, TX 78235-5301. The adenosine agonist ClAdo preferentially inhibits the Ca^2+-dependent component of glutamic acid release. However, exchanging Glu out of the cytosolic pool with 300 µM D-aspartate, 300 µM L(+)-APB reduced the remaining Ca^2+-dependent Glu release from 47.4 ± 3.1 to 29.8 ± 3.7 pmol/min/mg protein. Under the same conditions L(+)-APB had no significant effect on Glu or dynorphin A(1-8) release from rat MF synaptosomes.

441.0 ADENOSINE INHIBITION OF GLUTAMATE AND DYNORPHIN RELEASE FROM HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. L.P. Cannon, R.L. Cannon, C.L. Gannon, L.T. Byrnes, B.J. Frost. Queen's University, Kingston, Ontario K7L 3N6. The saw-whet owl (Aegolius acadicus) is a nocturnal raptor which acoustically locates its prey with great accuracy. The auditory localization abilities of this species has been studied using head orientation responses to sounds presented in a free field. Employing the search coil technique (Robinson, 1963; Knudsen et al., 1979) permitted the tracking of lateral and vertical head movements in response to playback sounds of mice moving through leaves. Saw-whet owls showed the greatest accuracy for sounds emanating from a region directly in front, spanning 40 degrees in each dimension. The average error was less than 1 degree in azimuth and just above this in elevation. Acuity was reduced at the extreme limits of the sound field, the mean error for speakers positioned at 30 and -30 degrees on either side being 6 degrees. A decline in accuracy for sounds originating from more than 10 degrees above the owl's interaural axis may be a function of the biological irrelevance of the particular stimulus at these elevations.
441.3 THE RELATION BETWEEN VISION AND SOUND LOCALIZATION ACUITY IN HAMMERS AND RATS. S. L. Sally* and J. B. Kelly. Lab. of Sensory Neurosciences, Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606.

It has long been noted that one function of hearing is to direct the eyes to the source of a sound. Recent analysis suggests that this function may be the major source of selective pressure on sound localization acuity in mammals.

If the ears are to direct the eyes to the source of a sound, then the question arises as to how accurate the ears must be to direct the eyes. We have shown that the acuity of sound localization needed to direct the eyes is not related to the size of an animal's field of best vision.

Using retinal ganglion cell counts we defined the size of the 'best vision' (i.e., their fovea) to the source of a sound. We reasoned that the acuity of sound localization needed to direct the eyes must be related to the size of an animal's field of best vision.

We measured the ability of human listeners to localize white noise bursts (150 msec duration) presented at horizontal and vertical locations distributed throughout nearly 360° of auditory space. Each subject reported the location of the sound source by orienting toward what he thought was the source. This head position was monitored with an electromagnetic device. Each sound source location was tested 5 or more times. Subjects could effectively discriminate among source locations separated by horizontal and vertical steps of 10° throughout most of the frontal half of space. Localization was most precise in the midline sources (standard deviations around 2°), but the precision of localization typically fell by no more than a factor of 2 for locations located from 150° within 35° of the horizontal plane. Front/back errors were infrequent. Localization precision was substantially lowered for sounds presented behind the subject; in part, this may reflect the difficulty in turning to face a sound presented from behind.

(Submitted by NIH grant NS 17850)

441.4 LOCALIZATION OF UNDERWATER SOUND IN THE CLAWED FROG, XENOPUS LAEVIS. R. J. Kelly* and J. B. Kelly. Lab. of Sensory Neurosciences, Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Cells in the superior olivary complex of the adult albino rat were selectively destroyed by microinjections of kainic acid. Following a 2-3 week recovery period, the binocular responses produced by the auditory nerve were examined using microelectrode mapping techniques. Rats were anesthetized with Equithesin (3.0 ml/kg i.p.), the cortical surface was exposed by craniotomy, and tungsten microelectrodes were made. Pure tone pulses were delivered to each ear independently through sealed loudspeakers fitted to specula inserted into the external meatus. Sound pressure levels were determined by probe tube measurements with a lyman type thermometer.

Cells selected for study were tested using binocular stimulation. Complete unilateral olivary ablation eliminated peripheral afferents as a source of binocular interactions. The majority of neurons exhibited binocular interaction at interaural intensity differences (IID) which were within the normal range. Maps of auditory cortex ipsilateral or contralateral to the lesion showed the same pattern of results. Complete suppression of monaural responses was observed in all cases. The results suggest a major role for the superior olivary complex in sound localization, but further evidence is needed.

The research was supported by NSERC operating grant 7571 to J.B.K.


The barn owl uses interaural time differences (ITD) for localizing the azimuthal position of sounds. Neuronal selectivity for ITD first appears in the avian n. laminaris and improves in the central nucleus 'core' (ICc) and the external nucleus (ICx) of the inferior colliculus. In the cat, ITD selectivity peaks at around 1° (Young and Rubel, 1983) and ICc neurons respond maximally not only to one particular ITD (the characteristic delay), dt, but also to dt n, where n is the time period and an integer. With the increase in phase ambiguity, does not occur when ICx neurons are stimulated with noise.

Isotopically applied bicusculline methodide (BMI, a selective GABA, antagonist) decreased the ITD selectivity of ICx neurons. The effects were identical for tone- and noise-evoked responses. In ICx, BMI decreased ITD selectivity to tones only in neurons tuned to frequencies below 5 kHz. BMI led to loss of ability of ICx neurons to signal unique information. In ICc neurons, BMI was able to decrease ITD selectivity to below 5 kHz, and eliminates phase ambiguity in ICx by interaction between the converging frequency bands. (Submitted by NIH and University of Colorado Foundation.)
441.9
BINAURAL INTERACTION IN THE BRAINSTEM AUDITORY EVOKED RESPONSE OF THE RABBIT (NESTELLA PITTIGLIO), G.L. Kavanagh, J.B. Kelly and T.W. Picton (SPON: R. Peterson). Lab. of Sensory Neuroscience, Carleton University, Ottawa, Canada, KIS 5B6 and Research Institute, University of Ottawa, Ottawa, Canada.

The binaural interaction on the BAER was examined in nine adult male ferrets. Animals were anesthetized with pentobarbital sodium (mg/kg I.P.), placed within a plastic-padded chamber, and the head was immobilized using a nontraumatic nose clamp. Clicks were produced by a 0.1 msec square wave, amplified and transduced by earphones over a broad range of intensities (34 to 104 dB peak SPL). Ear-electrodes were placed over the vertex and left and right mastoid. Responses were amplified 10,000 times, bandwidth filtered between 0.3 Hz and 10 kHz and averaged at a rate of 30 sweeps/sec. The analysis epoch for each channel was 10.24 msec and all responses were based on 1,000 sweeps. The binaural interaction component of the BAER was derived using the procedure of Dobie and Berlin (1979). At each intensity the responses to left and right monaural stimulation were summed to obtain a predicted binaural response. The predicted response was then subtracted from the binaural response generated by stimulating both ears simultaneously. This yielded a difference trace containing the binaural interaction component. Binaural interaction in the ferret BAER is characterized by a prominent negative wave with a latency similar to the fourth vertex-positive potential (V4). The wave showed an increase in latency and reduction in amplitude as sound intensity was reduced over a 70 dB range.

441.11

Barn owls use interaural differences in sound pressure level (ISPL) to compute locators in the source in elevation. I investigated the origin of neuronal selectivity for ISPL in the medial shell of the central nucleus of the barn owl, given that the auditory cortex of this species has an input stage to spatial elevation coding in the external nucleus (IXe).

Anatomical connectivity was demonstrated by retrograde labelling with horseradish peroxidase. Neurons of IXe receive direct massive projections from the contra-lateral nucleus laminaris (NL), nucleus laminaris anterior lateralis pars posterior (VLVp), lateral region of the superior olive, and sparse projections from contra-lateral MS.

All above nuclei show stimulus-response curves sensitive to ISPL and generally insensitive to interaural time difference (ITD), culminating in typically very sharp "E" response curves in MS neurons: they are excited by ISPL's favoring the contralateral ear, and inhibited by ISPL's favoring the ipsilateral ear. Preliminary data from liddocaine injections into the projecting nuclei suggest that both VLVp and MS make inhibitory contra-lateral connections. This is consistent with predictions based on the model of connectivity and ISPL-response character.

441.13

Single units in the ventral nucleus and Po of barbiturate anesthetized cats were studied using free field stimulation. A series of tone bursts, broad band (BBN), or band pass (BBP) white noise bursts which systematically varied in level was delivered from each of several azimuths. Isolate contours were computed and plotted in SPL or spectrum level vs. azimuth coordinates. On the basis of these contour plots, three classes of response patterns were distinguished. 1) Non directional. These units were broadly tuned to azimuth for BBN, BBP, and tones. 2) Azimuth-selective, bandwidth-insensitive. These units showed a sharp peak near zero degrees of azimuthal selectivity and discharge rate was similar for BBN, BBP, and tones. 2-tone stimulation resulted in little evidence of sideband interaction. 3) Azimuth-selective, bandwidth-sensitive. These units displayed highly directional tuning to BBN with decreasing selectivity as the spectral bandwidth narrowed. Units often exhibited broad azimuthal tuning to total stimuli, however some units were more directionally tuned to some frequencies than others. Unit discharge rate was lowest to broadband stimulation, and highest to narrow band stimulation centered within the frequency response area suggesting the existence of inhibitory sidebands.

Two-tone stimulation showed direct evidence of inhibitory and facilitory sidebands, the former being much more common. Some units displayed inhibitory sidebands on both sides of the excitatory response area while others exhibited only one inhibitory sideband. The degree of sideband inhibition appears to be a function of both stimulus level and direction. Sideband inhibition appears to play a role not only in shaping a unit's frequency selectivity, but also its directional selectivity. (Supported by NINCDS Grant 17220 and BRSG S07RR05373)

441.12

Several nuclei in the auditory pathways that receive input from both ears project to the inferior colliculus (IC). These include the nucleus of the superior olive, lateral lemniscus, and the auditory cortex. Several of these nuclei project to the same region of the IC, but do these projections converge on the same neurons? Here we report that single neurons in the IC receive convergent inputs from the same binaural sources.

We recorded from neurons in the IC of the unanesthetized rabbit that were sensitive to ITD's of the waveform or the envelope. Calibration stimuli included tonal and broadband noise. The tonal stimuli were modulated/demodulated tones were delivered dichotically. Responses were assessed at several tonal or modulation frequencies.

Neurons sensitive to ITD's are thought to arise from the convergence of phase-locked signals from the two ears along pathways which have fixed delays. Such a mechanism predicts a linear relationship between the interaural phase difference to which a neuron is most sensitive (the mean phase) and the stimulating frequency with the slope representing the difference in delays from the two ears. We have observed significant deviations from this expected linearity in the IC. These deviations can take many forms. They can appear as oscillations of the mean phase around the regression line, or as a separation of the mean phase vs. frequency plot into two segments with different slopes. The two slopes indicate that the neuron receives convergent input from two sources, each of which processes a different ITD over a different range of frequency. Responses can also exhibit nulls at intermediate frequencies. All of these effects suggest convergence from two or more sources, each of which in turn receives phase-locked inputs from the two ears.

This research was supported by NINCDS Grant NS-18027.

441.14

In the barn owl, sensitivity to interaural time differences arises in nucleus laminaris (NL), the first recipient of binaural input. Eight nerve afferents synapse upon the neurons of the magnocellular cochlear nucleus (NM), which in turn project bilaterally to NL. The ipsilateral axons from NM enter NL dorsally, while the contralateral axons enter ventrally. The dorso-ventral arms created by the interdigitation of these afferent form maps of interaural time difference.

At the ultrastructural level, the owl's time coding pathway resembles that of the chick. Each neuron in NM receives two types of synaptic input. One is the caliciform ending typical of eighth nerve fibers. These profiles contain round clear vesicles, and form a large number of punctate asymmetric synapses. The other type of profile is a modified type I dendrite packed with ellipsoid vesicles. These profiles may correspond to the GAD positive terminals observed with the light microscope.

The afferent axons from NL are divided into two types, one within NL, as well as many large club shaped terminals upon the dendrites and somata of NL neurons. Each NL neuron has a large oval soma, about 30µm in diameter, and short thick dendrites. Some of NL neurons receive thick punctate synaptic densities from NL terminals and a second type of profile which forms narrow single densities (Parks et al., 1983) J. Comp. Neurol. 214:32-42). NL neurons, the large (10µm) axon becomes myelinated at the soma, and membrane adjacent to the origin of myelination does not contain a dense undercoating.

Supported by NIH grant R29 NS25507.

The spatial sensitivity of single neurons and auditory space representation in the primary auditory cortex of Equus tercicrustocephalus was studied under free field stimulus conditions. A 4 ms pure tone pulse was emitted from a loudspeaker 23 cm in front of the bat to determine the best frequency (BF) and minimum threshold (MT) of each neuron. Then an FM signal sweeping one-octave downwards across the neuron's BF was delivered as the loudspeaker was moved through the frontal auditory space to determine the spatial response center at which the neuron's MT was the lowest. The stimulus was then raised 10-20 dB above each neuron's lowest MT to measure the spatial response area. All response centers are located in the contralateral frontal auditory space (0° and 50° in azimuth, 0° and 25° in elevation). Response centers tend to move toward the midline and slightly downward with increasing BF. Since high BF neurons are located anteriorly and low BF neurons posteriorly, the auditory space appears to have an orderly representation in the auditory cortex along the tonotopic axis. Thus, the lateral space is represented posteriorly and the middle space anteriorly. While the response area of cortical neurons expands asymetrically with stimulus intensity, high BF neurons tend to have smaller spatial response areas than low BF neurons. The BFs, MTs, spatial response areas and response centers of neurons sequentially isolated from an orthogonally penetrated electrode are similar indicating columnar organization in the auditory cortex.


Three starlings were tested in a GO/NOGO-procedure on their ability to detect changes in the duration of a tone. Stimuli were pure tones at three frequencies (4 kHz, 2 kHz, 0.5 kHz) and three standard durations (800 ms, 200 ms, 100 ms) (Table 1). Amplitude was randomized in steps of 6 dB from 0 to 20 dB SPL. Increments and decrements in duration (Δ T) ranged from 5% to 90% of the standard duration. In the range of the presumed threshold they varied in steps of 5% of the standard duration. Stimuli of different durations were presented by the method of constant stimuli. Thresholds were determined using signal detection theory, threshold criterion was the discrimination measure d' of 1.8. For an increase in duration no influence of frequency on the discrimination threshold was found (Table 2). Weber fractions Δ T/T ranged from 0.12 at 800 ms standard duration to 0.23 at 100 ms standard duration. For a decrease no influence of frequency on the discrimination threshold was found (Table 2). Weber fractions Δ T/T ranged from 0.05 - 0.1, which coincides with the acuity of tone discrimination in non-mammalian vertebrates. This accuracy is considerably greater than found earlier for frequencies above 1 kHz (ΔT/L-0.45), which may reflect the fact that a tonotopic organization is found in the amphibian but not the basilar papilla of frogs.

441.18 UNILATERAL NEONATAL DAMAGE OR RESULTANT CORTICAL PATHOLOGY. Nathan T. McMullen, Bruce P. Greenberg, and Edmund M. Glaser. Dept. of Physiology, Univ. of Maryland Sch. of Med. Balt. MD 21201.

In recent years we have demonstrated unilateral cochlear damage in the neonatal rabbit results in substantial alterations in presumptive target neurons in the contralateral auditory cortex (McMullen et al, JCN, 207: 92-106, 1988; Glaser et al, Lab. Brain Res. 1988). Based on 3-D reconstruction and quantitative analyses of 100 Golgi-imregnated neurons obtained from 4 neonally deafened and 2 control rabbits, we report that spine free nonpyramidal cells ipsilateral to the neonatally damaged cochlear exhibit a characteristic pattern of dendritic expansion (ca. 24% increase in total length) and abnormally recurved dendrites (42% of cells). The expansion was due entirely to increased branch length as there was no change in the number of dendritic branches. Recurred dendrites were almost exclusively directed tangentially or toward the white matter. These data indicate that unilateral cochlear damage results in the reorganization of both ipsilateral and contralateral ascending auditory pathways. The failure of the unamaged ear to maintain normal cortical dendritic growth bilaterally is evidence that binaural competition is an important component of auditory cortical maturation. Supported by NIH grants NS17861, RR01169 and the Deafness Research Foundation.
442.1 CORTICAL DEAFNESS CANNOT ACCOUNT FOR "SENSORY APHASIA"

In Japanese macaques, absence of the lesion to M.E. Hutchings* and J.S. Kroll*. (SPON: C. Blakemore).

sensory systems: auditory systems VIII

University Laboratory of Physiology, Parks Road, Oxford

The right bullae of anaesthetised gerbils were


removed using a scalpel. The round window was

University Laboratory of Physiology, Parks Road, Oxford, England. Otitis media with effusion (OME) is the

University Laboratory of Physiology, Parks Road, Oxford, England. Otitis media with effusion (OME) is the

Middle ears inoculated with bacteria were inflamed and

Middle ears inoculated with bacteria were inflamed and

contains non-viable H. influenzae type b Kagan (1-3 x 10^9/ml). Animals were sacrificed at intervals and the temporal bone was decalcified, sectioned and examined. Middle ears inoculated with bacteria were inflamed and contained serious effusions which resolved by 14 days post-operative. The round window was thickened and polymorphonuclear leucocytes were present in the scala tympani by one day post-operative and in the scala vestibuli by three days. Middle ear pathology included the formation of granulation tissue and new bone. The mucoperiosteum remained elevated one month post-operative. Saline inoculated ears developed scant effusions and the tympana were normal by 5 days post-operative.

442.5 GENERALIZATION OF CONDITIONED SUPPRESSION IN RATS FOLLOWING SALICYLATE INDUCED TINNITUS. J.F. Brennan and P.J. Jastreboff, Dept. of Psychology, University of Massachusetts Boston, Boston, MA 02125.

The extent of auditory stimulus control of lick suppression was studied in naive rats. Extracts of 36 piggledy rats exposed to daily injections of sodium salicylate, 350 mg/kg, induced either before or after acquisition training. Nine successive daily injections resulted inlick training, acclimation exposure to the conditioned stimulus (CS), suppression acquisition sessions involving 5 onsets of 1-min 10-kHz CS + 1 mA footshock, and 5 extinction tests wherein the CS frequencies varied randomly among 1-8, 2-9, 9-10, and 11 kHz (all at 60-dB). In Experiment 1, 16 subjects were exposed wussily to a 7-kHz tone superimposed upon noise of 60-dB, except for the CS probes when only the respective tonal value was presented. Control rats were exposed to the 7-kHz background only. Stimuluscontrol was evident in the saline injected control groups of both experiments by the absence of auditory onset of suppression obtained within individual subjects. Both groups injected after training showed little auditory control and rapid extinction. The saline probes injected before training showed severe suppression and distorted gradients reflecting a summation effect. These data are consistent with a model that infers the differential presence of tinnitus. (Supported by NIDR 1988)

442.6 REDUCED SUSCEPTIBILITY OF THE COCHLEA TO REPEATED EXPOSURE BL Lonsbury-Martin, DJ Franklin*, BB Stagner* and GK Martin*. Dept. Otolo-

Susceptibility of the cochlea to repeated exposure was investigated in 4 rabbits with measures of behavioral thresholds and distortion-product emis-

sibility to repeated exposure. [NS10940, ES03500, DRF]
SENSORY SYSTEMS: AUDITORY SYSTEMS VIII

Physiology, Univ. of Tuebingen, Morgenstelle 28,
was taken at 20 m in intervals for analysis by high pressure liquid chrom-
443.1
442.7
442.8
continuation. Response rates were between 2400 and 3000/hr. Extracellular
the animals were allowed to self-stimulate for 1 hr while sampling con-
ipsilateral to the stimulation electrode, and a series of baseline samples
hypothalamic (PFH) stimulation. A microdialysis probe was inserted into
the posterior accum bens through a previously implanted guide cannula
EFFECTS OF BILATERAL ABLATION OF THE AUDITORY CORTEX
AND/or CINGULATE CORTEX ON THE BIOSONAR BEHAVIOR OF
THE MUSTACHED BAT. S. J. Gubisch*, N. Saga, and H. Riquiermard*, Dep. of
Biophysics, Washington Univ., St. Louis, MO 63110.
The mustached bat (Pteronurus parnellii) adjusts its complex biosonar
vocalization center, the cingulate cortex (Cg), contains a motor map
for this cholinergic action.
These neurons utilize delay lines created by sub-thalamic neurons tuned
increases; 3) it lengthens pulse CF duration when it detects a flying insect; 4)
it then decreases pulse duration and increases pulse repetition rate. The
mustached bat's auditory cortex (AC) has several functional subdivisions for
processing different acoustic features. One such division is the central
vocalization center, the cingulate cortex (Cg), contains a motor map
which was swung towards a larger target. The bats were then retested
following large bilateral ablations by aspiration of the Cg, Cg followed by
AC, AC, or DSCF area (part of the AC). The Cg ablations had no
measurable effect on any of these behaviors. In contrast, following AC
ablution, the amount and stability of DSC significantly decreased, and reaction
time increased. These RPs became more variable post-ablative, and the
RPs also showed a large transient increase immediately following each
pendulum test session. The DSCF-ablated bats showed changes in DSC similar to
those displayed by the AC-ablated bats. In conclusion: 1) the AC is
involved in stabilization of the RP, and its DSCF subdivision is involved in
"fine-tuning" of DSC; 2) the role of the Cg in biosonar behavior is enigmatic.
(Supported by AFOSR grant 486-NL-192).

442.9
442.10
ICV INJECTION OF CARBACHOL SUPPRESSES ACOUSTIC STARTLE RESPONSES-
The acoustic startle response is a simple behavi oral model for studying the neural mechanism
underlying the transformation of a sensory input into a motor output. Several transmitters were
found to modulate this short latency reflex. Since systemic injection of acetylcholine (Ach)
antagonists blocks the startle response, and since an early relay station in the startle circuit,
the cochlear nucleus, is cholinergic, the present study investigated the role of Ach in
modulating the startle response at the cerebral level. Microinjection of 500ng carbacho (Ach
agonist) into the lateral vestibular yielded a significant suppression of the startle related
EMG-amplitude of the temporals muscle. ICV injection of 500ng atropin (Ach antagonist)
showed a slight tendency for enhancing the EMG-amplitude. These results suggest that Ach modulates
the motor response to a high intensity acoustic stimulus. Preliminary data indicate that the
last relay station in the startle circuit, the motor trigeminal nucleus, is not the target site for
this cholinergic action.

443.2
DOUBLE-LABEL DEOXYTUCOSIO AUTORADIOGRAPHIC STUDIES OF UNILATERAL AND BILATERAL MEDIAL FOREBRAIN BUNDLE SELF-
STIMULATION IN RATS. R. F. Ackermann and M. E. Phelps. Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.
We are studying intracranial self-stimulation (ICSS) responding with [F-18] Fluorodeoxyglucose (FDG) and [C-14] 2-deoxyglucose (2DG) double-label autoradiography in rats having chronic bilateral medial forebrain bundle (MFB) electrodes. Double-label autoradiography allows visualization of two conditions in essence: (a) double-label autoradiography, (b) autoradiography of rads

443.1
MICRODIALYSIS SHOWS INCREASED DOPAMINE TURNOVER IN THE NUCLEUS ACCUMBENS DURING LATERAL HYPOPHYSIAL SELF-
Extracellular dopamine (DA), and its major metabolites dihydroxy-
phenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured in five rats trained to lever press for a fixed current of perifornical lateral hypothalamic (PFH) stimulation. A microdialysis probe was inserted into the posterior accumbens through a previously implanted guide cannula ipsilateral to the stimulus electrode, and a series of baseline samples was taken at 10 minute intervals. Microdialysis was performed by liquid chro-
atography with electrochemical detection. After DA levels had stabilized, the animals were allowed to self-stimulate for 1 hr while sampling continued.
Response rates were between 2400 and 3000/hr. Extracellular levels of DOPAC and HVA increased significantly, indicating an increase in DA turnover that persisted beyond the period of self-stimulation by about 40 min. Extracellular dopamine also increased. These results demonstrate an increase in DA turnover in the accumbens asso-
ciated with self-stimulation in freely moving rats. Stimulation of the PFH that induces feeding has also been shown to increase extracellular DA. DOPAC chromatograms on another study (1) which suggests that DA turn-
over in the accumbens is involved both in the reinforcement of PFH self-
stimulation and the induction of food intake.

MOTIVATION AND EMOTION I
443.3

TWO PROPERTIES OF THE INTEGRATOR FOR REWARDING BRAIN STIMULATION. P. Gourineau, B. Walkert and K. Patterson. School of Psychology, University of Ottawa, Ottawa, Canada. KIN 6N5.

Four experiments evaluated the effect on self-stimulation frequency thresholds of altering baseline activity of the directly stimulated reward substrate. Baseline activity was altered by a continuous, low frequency of stimulation pulses while the rats pressed a lever to earn bursts of lateral hypothalamic stimulation at the same location (0, 2, 5, 10 or 20 Hz) decreased frequency thresholds by its own frequency when it was applied to the same electrode (Expt 1) and by frequencies predicted by empirically determined summation levels when it was applied to a contralateral electrode (Expts 1 & 2). The effect was additive over a wide range of baseline activity (Expt 3) but it was completely eliminated when the continuous stimulation was restricted to times between response-triggered bursts (Expt 4). We conclude, first, that the reward integrator defines an absolute threshold in the sense that it is not sensitive to baseline activity and, second, that the integrator accepts input only for the duration of the response initiated train.

443.4

INVolVEMENT OF DOPAMINE D2 RECEPTORS IN THE REINFORCEMENT OF OPERANT BEHAVIOUR. S. Nakajima. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

Previously I have reported that a dopamine D1 receptors are involved in the reinforcement. Now the effect of dopamine D2 receptor blockade on operant behaviour was examined using raclopride.

In the rats trained to press a bar to obtain food, injection (IP) of raclopride suppressed responding in a dose related manner. Another group of rats were trained to press a bar for electrical self-stimulation of the ventral tegmental area. Raclopride again suppressed responding in a dose related manner. Compared with food-reinforced responses, about 10 times higher doses of raclopride were required to suppress self-stimulation. Responding for stimulation at 160 Hz was more easily suppressed than responding for 60 Hz. Injection of raclopride into the nucleus accumbens suppressed self-stimulation of the ipsilateral ventral tegmental area (ED 50 = 10μg).

Blockade of dopamine D2 receptors seems to interfere with the brain mechanism of reinforcement. (Raclopride was a gift of Astra AB, Sweden)

443.5

EFFECTS OF ANTERIOR MEDIAL FOREBRAIN BUNDLE LESIONS ON SELF-STIMULATION OF THE LATERAL HYPOTHALAMUS AND VENTRAL TEGMENTAL AREA. B. Murray and P. Shiggal. CSBN, Concordia University, Montreal, Quebec H3C 1M8.

Psychophysical data suggest that reward fibers directly link the lateral hypothalamus (LH) and ventral tegmental area (VTA). As a step toward identifying the nuclei from which these fibers arise, we assessed the effect on self-stimulation of both the LH and VTA of electrolytically lesioning the anterior medial forebrain bundle (MBF). Changes in the rewarding effect were inferred from lateral displacements of rate-frequency functions. Lesions in 5 of the 7 rats displaced the rate-frequency functions for the LH and/or VTA sites toward higher frequencies (26-37 Hz above baseline), an effect consistent with a decrease in the rewarding impact of the stimulation. The ineffective lesions were restricted to compartment 'c' of the anterior MBF (Nieuwenhuys et al. J. comp. Neurol. 1982, 206, 49) while the five effective lesions invaded the more lateral compartments, 'a', 'd', and 'e'. Thus, the shifts in the rate-frequency functions may have been caused by damage to neurons projecting through compartments 'a', 'd', and 'e', or neurons with somata in the lesioned areas of the anterior lateral MBF. Although these shifts could also have resulted from damage to ascending dopaminergic fibers, it is not clear that the distribution of these fibers is consistent with the differential effect of the medial and lateral lesions.

443.6

MODULATION OF COLLISION EFFECTS BY LESIONS: IMPLICATIONS FOR IDENTIFYING NEUROTRANSMITTER SUBSTRATES. B. Murray and P. Shiggal. CSBN, Concordia University, Montreal, Quebec H3C 1M8.

Lesion methods have long been used to identify the neurons responsible for the rewarding effects of electrical brain stimulation. Unfortunately, the anatomical interpretation of such data is ambiguous. A decrease in the rewarding effect could be due to damage to the directly stimulated neurons, their efferents, or neurons that modulate transmission in the reward circuit. In contrast, a lesion effect inferred from psychophysical data is consistent with a clear anatomical interpretation: The tip of the two stimulation electrodes, in going through the neural test track of a common population of reward neurons. By combining collision tests with lesions, we hoped to render the anatomical interpretation of lesion data less ambiguous. We reasoned that if a lesion destroyed some of the reward neurons undergoing collision, then the collision effect would decrease in size.

Trains of pulse pairs were applied to the LH and VTA, with each electrode delivering either the conditioning (C) or the test (T) pulses. Collision was inferred from an abrupt decrease in stimulation effectiveness as the C-T interval was decreased. In 2 of the 4 rats, a lesion aimed 1.5 mm anterior to the LH site decreased the collision effect by 25% to 30%. The simplest interpretation of these data is that the lesions damaged reward fibers directly linking the two sites.

443.7


Male rats (350g) were implanted with monopolar stimulating electrodes (Plastics One, M1.5, 0.5V D/V to 83 to dura) and in the anterior VTA (A4.0;5.7;8.7) and in the anterior VTA (A4.0;5.7;8.7). Animals were trained to lever press for 0.5sec trains of 0.1sec cathodal pulses at both electrodes. The two trains were systematically varied allowing identification of the frequency of stimulation which produced half maximal rates of lever pressing at a number of currents for the two trains. Self-stimulation thresholds (300μA/20sec) were made at the VTA electrode. Frequencies necessary to produce half maximal responding at the same currents were again used for the LH electrode. A range of 0.9 log units (as measured by the rate/frequency function (Gallitell et al. Psych Revs. 88, 228-273)). This was performed in light of Bisajew & Shiggal's results (J Neurosci. 6, 919-929) suggests that a large proportion of the reinforcing signal generated by the LH electrode parasite from the VTA.

This work was supported by Grant NSF BNS86-19759.

443.8


The respective roles of intrinsic neurons and a dopamine containing nigrostriatal system in mediating loss of locomotor activity in the lateral hypothalamus and in the striatum were examined. Previous studies demonstrated the locomotor effect (expt 1) and in the hypothalamus was found to be dose related (expt 2).

The dopamine depletion was produced using 6-OHDA (6-hydroxydopamine) treatment (20μg/μl) in the striatum and in the lateral hypothalamus. The lesion produced a marked reduction in locomotor activity in both structures. The dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 3, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 4, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 5, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 6, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 7, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 8, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 9, the dopamine content of the striatum and in the lateral hypothalamus was reduced by a factor of 4 (6-OHDA) at 2 weeks post-treatment. In expt 10, the dopami...
MOTIVATION AND EMOTION I

which blocks the effects of post-synaptic dopamine

Montreal, Canada H3G 1M8.

FUNCTION BY 0.6 LOG UNITS. R.A. Wise and P.P. Rompré
demonstrated electrophysiologically with several D-2

inactivation might account for the precipitous nature of

extinction of responding for brain stimulation reward, we

assessed the effects of the D-l antagonist SCH 23390,

that the precipitous failure of self-stimulation under

response under SCH 23390 is consistent with the hypothesis

completely; this is twice the maximum shift seen with the

transmission in the dopaminergic link in the reward

system. Opioids have rewarding actions both near the

terminals. That nucleus accumbens (NAS) opiates should be

near the dopamine cell bodies but not near the dopamine

in the region of dopamine terminals in nucleus accumbens;

the potency of opioids in NAS seems less than that

reported for ventral tegmental morphine injections.

EFFECTS OF NALOXONE ON REWARD INDUCED BY DORSAL RAPHE
ELECTRICAL STIMULATION: A STUDY USING THE CURVE-SHIFT
Department of Psychology, Concordia University, Montreal,
Quebec, Canada H3G 1M8.

The present experiment examined the effects of several
dooses of naloxone on bar-pressing for dorsal raphe electro-
cal stimulation. After stabilization of self-stimulation, rats
tested on different days with vehicle-saline and three
dooses (2, 4, and 8 mg/kg) of naloxone administered in

either an ascending or descending order of dosage. Curves
relating bar-pressing rates to the stimulation frequency
were obtained five times just before drug injection, and

repeatedly 0 to 90 min after drug injection. Naloxone

shifted the curve to the right, increasing frequency
thresholds for dorsal raphe stimulation. The increase in

threshold was not dose-dependent, the largest shift was

observed (0.15 log unit) at 4 mg/kg, 60 to 90 min after the

injection. Because naloxone reduced the maximal response

rates by less than 10%, we inferred that the shift in

frequency threshold was due to a decrease in the rewarding

effectiveness of the stimulation. No significant difference

was found between shifts in threshold obtained when the

drug was administered in an ascending or a descending order
d of dosage. These results show that like rewarding stimula-
tion of the medial forebrain bundle, rewarding stimulation

of the dorsal raphe is inhibited by blockade of opiate

receptors.

DEPOLARIZATION INACTIVATION OF A9 OR A10 DOPAMINE NEURONS
BLOCKS BAR-PRESSING FOR MESENCEPHALIC DORSAL RAPHE
Department of Psychology, Concordia University, Montreal,
Quebec, Canada H3G 1M8.

Previous experiments have shown that systemic piomoxide
and ventral tegmental area (VTA) morphine can induce
dopamine depolarization inactivation; this results in a
complete suppression of responding for central gray
brain stimulation reward (BSR). We now report the effects
of systemic piomoxide and morphine injections in either VTA
or substantia nigra (SN), on medial forebrain bundle BSR.
Estimates of BSR threshold were obtained using the curve-

shift paradigm under different drug treatments. At high

doses (2.5-5.5 mg/kg) naloxone in either the saline or

VTA produced a significant decrease in BSR threshold. Higher
dooses (5-10 mg/kg) of morphine given after a systemic injection
of piomoxide completely blocked bar-pressing. The behavior
was reinstated with either central injection of muscimol (12.5-

25 mg) or systemic biclofen (0.5 mg/kg). Because similar
treatment with DAGO abolished the rewarding properties of

depolarization inactivation, we infer that blockade of bar-

pressing after systemic piomoxide and central morphine was
due to blockade of dopamine cell firing.

CHRONIC NEUROLEPTIC TOLERANCE AND SENSITIZATION IN THE
CURVE-SHIFT BRAIN STIMULATION REWARDED PARADIGM. M.L. Lynch
and R.J. Carey, VA Med Ctr & SUNY BRC, Syracuse, NY 13210.

Chlorpromazine (Hal) induced catalepsy shows a tolerance with chronic treatment rather than sensitization which
would be predicted by depolarization blockade. Depolarization of
delayed onset clinical effects. The present study was con-
ducted to examine tolerance vs sensitization of motor vs
"reward" effects in the curve shift paradigm. Acute 0.07
mg/kg Hal produced reliable increases in threshold for 100
Hz biphasic square wave stimulation (0.1sec pulses, 0.1sec
interval, 0.2sec train) to the VTA (platinum electrodes).
Stimulation was begun at maximum intensity and decreased by
5% every 3 min., with a 2-min time-out. N=4 rats received
Hal 1 hr before testing. Group 1 received sequential 20, 40, 60, 80,
90, 100% post and 4-min vehicle pre. The latter two groups showed consistent rate-intensity functions over test days. The pre group showed tolerance to the trace bar-pressing effect but
there is a suggestion of sensitization for rate suppressing
effects in the asymptotic range. Reversing pre and post
conditions argued against environmentally-specific toler-
ance. HPLC-EC of DA and its metabolites on chronic injec-
tion days 36-37 revealed partial tolerance to acute Hal-
induced stimulation of DA turnover in both pre and post
drug groups. Tolerance was evident from DOPEG/DA and HVA/DA
ratios but not 3-MT/DA. These findings question the rele-

ance of threshold shifts for antipsychotic efficacy and
emphasize the need for assessing mechanisms of chronic ad-
aption processes for different behavioral effects.
443.15

**MOETIVATIONAL EFFECTS OF NEUROLEPTICS, H. M. Geyer III, M. Contelletta, V. Ramirez, R. Corbett, and S. Fielding.**


A progressive fixed ratio (FR) schedule of milk reinforcement for nose-poke responses was used to assess the effects of neuroleptics. It was hypothesized that motivational deficits would appear as the FR increased and motor deficits would be present across all FR's.

The rats on a restricted diet were pretreated with various doses of haloperidol or chlorpromazine, either with or without their nose-pokes for dippers of milk (0.02 ml) were recorded. The performance was not reduced by diazepam at 5 mg/kg or isoperimazine at 20 mg/kg, however, chlordiazepoxide at 0.02 mg/kg, chloridazine at 10 mg/kg, Sch 23930 at 0.005 mg/kg and chlorpromazine at 0.31 mg/kg all reduced responding. The increase in maximum FR's from 12 to 24 and 48 did not effect the performance of saline treated rats. However, chlorpromazine at 0.31 mg/kg reduced the responding at FR 48 but not at FR 12, consistent with a motivational deficit and not motor impairment. Other neuroleptics have shown similar FR dependent decrements in performance, consistent with the clinical reports of neuroleptic induced "anhedonia". The test appears to provide a method for measurement of motivational changes induced by pharmacological agents.

443.17

**HYPOTHALAMICALLY ELICITED FEEDING AND SELF-STIMULATION: TWO MODELS OF INTEGRATION OF THE SAME OUTPUT.**

M. Waraczynski and J.M. Kaplan*. Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104

Many perifornical hypothalamic electrodes that support self-stimulation also elicit feeding. Refractory period and conduction velocity estimates for electrode placements and elicited feeding, using bar press rate and ingestion rate, respectively, as dependent measures. For self-stimulation, increases in intensity shift the frequency-response curves to the left without changing asymptotic performance level. For elicited feeding, increases in intensity elevate the asymptotic ingestion rate, but do not appreciably change the location of the curve along the frequency axis. The qualitatively different parametric profiles underlie rather different integrative processes, acting on output from a common substrate, mediating the respective behaviors.

Supported by grants M095347 (AM) and NS08094 (JMK) from the National Institutes of Health.

443.16


Dopamine (DA) is accepted as playing an important role in lateral hypothalamic self-stimulation reward, but it is unclear if DA carries or modulates this reward signal. Using the standard rate-frequency paradigm, we have confirmed that amphetamine (indirect agonist) increases LH reward in rats and that apomorphine (direct agonist) decreases LH reward. However, the argument that apomorphine disrupts normal DA LH reward signalling by producing non-contingent DA reward is not supported by our recent pilot work indicating that apomorphine microinjections into the nucleus accumbens increase LH reward in a dose dependent fashion on mouse lesion loci and on CCK dose (range, 1ng - 10ug).

Supported by Whitaker Foundation grant to J.S.

444.1

**THE INCENTIVE MOTIVATIONAL PROPERTIES OF FOOD AND OPIOATES ARE MEDIATED BY A COMMON BRAINSTEM SUBSTRATE.**

A. Becerra and D. Van der Kooy. Neurobiology Research Group, Anatomy Dept., Univ. of Toronto,Toronto,Canada MSG 1A8.

The reinforcing effects of opiates are dependent on both their pharmacological and drive reduction properties. Bilateral ibotenic acid lesions (.2 ul of a 4% solution on each side) of the tegmental pedunculopontine nucleus (TPP) abolished the morphine (2-10 mg/kg s.c.) conditioned place preference produced in opiate naive but not in opiate dependent (60 mg/kg/day for 14 days) rats. These results suggest that the TPP mediates specifically the incentive motivational properties of opiates. Moreover, the incentive motivational and aversive withdrawal properties of opiates can be measured separately by pairing a novel place with only with morphine in naïve rats and only with morphine withdrawal in separate dependent rats. TPP lesions blocked the place preferences for the morphine paired environment, but not the place aversions to the withdrawal paired environment.

A similar experiment was carried out in food deprived (Purina lab chow) rather than morphine as the reinforcer. TPP lesions did not block the food preferences produced in food deprived (24 hours) rats, nor did they block the place aversions of food deprived animals conditioned to a place paired with the lack of food. However, TPP lesions did block the place preferences produced by food in satiated rats. These satiated rats (TPP lesions and sham controls) did not eat the food in the training environment, but controls showed large place preferences for the food paired environment. We suggest that reinforcers can act through two parallel motivational mechanisms in the nervous system-a circuit through the TPP that mediates the impact of incentive stimuli, and an independent drive reduction mechanism processing homeostatic signals arising from within the organism.

444.2

**AVERSIVE PROPERTIES OF OPIATE RECEPTOR BLOCKADE: EXCLUSIVELY CENTRAL MEDIATION IN NAIVE AND MORPHINE-DEPENDENT RATS. TH. HAND, G.F. KOOB, I. STINUS, and M. LE MOAL. INSERM U.259 - Université de Bordeaux II, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex - France, and BCR1 - Scripps Clinic, La Jolla, CA 92037, USA**

The motivational effects of exclusively peripheral or central opiate receptor blockade were studied using place conditioning. Intraventricular (ICV) mexitelinaloxonium (MN) produced place aversions in both naive (200-1000 ng) and morphine-dependent rats (20-500 ng). Interestingly, the full withdrawal syndrome ("wet dog shakes", jumping, hyperactivity, teeth chattering, writhing, diarrhas, weight loss) was never observed, although some of these signs were occasionally seen in the dependent groups at highest dose. Subcutaneous MN (0.03-10 mg/kg) was ineffective in naive rats and produced place aversions in dependent rats only at the highest dose (a dose at which some central blockade may have occurred). These data suggest that the aversive properties of opiate receptor antagonism are centrally mediated in both naive and dependent rats, and that their enhancement in dependent rats results from a sensitized central mechanism rather than from the recruitment of a peripheral component. They also suggest that the so-called "withdrawal syndrome" may not be a suitable model for these aversive effects.
444.3
ESB OF LATERAL HYPOTHALAMIC FEARNFLIGHT SITES IN RATS PRODUCES CONDITIONED PLACE AVOIDANCE. D. Sacks and L. Parkes. (SPONSOR: K. F. Green) Dept. of Psych, Bowling Green State University, Bowling Green, OH, 43403.

Electrical stimulation of the brain (ESB) in the anterior lateral hypothalamic area (AHA) has been shown to produce behaviors indicative of emotional state (Williams & Korotkets, Brain Sco., 1987.) Following ESB of AHA stimulation-induced "fear" produces species-typical defensive and escape behaviors (Neurosci. Abst., 1987, #127,1), and the present study examined whether a conditioned place avoidance would develop in an environment in which the AHA-ESB was applied.

The experimental group (n=6 rats) received the AHA-ESB on their preferred side of the apparatus, and ESB was delivered in 40 minute training periods. The control group (n=6) experienced the same procedures but with no ESB. The animals' side preference was then reassessed.

After 4 pairings of the ESB with the previously preferred side of the test chamber, a reliable decrease in side preference was exhibited on the first test after training [F(1,10)=96.2, p<.001] as well as 3 days [F(10)=234.8, p<.001] and 7 days [F(10)=8.37, p<.001] afterwards.

This research demonstrates the ESB of the AHA generates an aversive affective state that produces strong avoidance of an environment in which such stimulation was received. These results support the contention that ESB of AHA sites which yield the unconditioned response of flight does generate a true emotional state resembling fear. Therefore, the AHA may be part of a neural circuit which mediates both the behavioral manifestations and internal experience of this emotion.

444.4

Prolonged electrical stimulation to rewarding brain sites will elicit escape behavior in rats. The present experiment was designed to determine if this escape behavior is reinforced by the nociceptive properties of the stimulation or reinforced by the rewarding effects of the onset of the next stimulus. If an analgesic drug having no effect on rewarding brain stimulation, lowers the threshold for escape from stimulation to rewarding brain areas, it would also suggest that nociceptive stimulation is nociceptive. In the present experiment we determined the effects of two drugs that met the above criteria, butylscopolamine (BSC) and naloxone (NX), respectively, on the threshold for escape from stimulation to the medial forebrain bundle-lateral hypothalamo (MFB-LH) area. Results indicate that BSC (6.5-10mg/kg) raises the escape threshold whereas NX (5.0-16 mg/kg) lowers the escape threshold, suggesting that aversiveness of electrical brain stimulation to the MFB-LH is the result of the nociceptive quality of stimulation and not the result of the rewarding effects of the onset of stimulation.

([Supported in part by NIDA Grant DA 00099 (CK) and a Culpepper Award (JP)].)

444.5

Aimless rats were placed in the test arena and in the absence of cat smell in rats of groups following damage to the main, accessory or both olfactory systems, following intranasal infusions of zinc sulfate (ZINC), transections of the vomeronasal nerve (VNX), or olfactory bulbectomy (OBX), respectively. Control group animals (CON) received either sham surgery or intranasal infusions of distilled water.

During days 1-5 of testing, half of each group (CON, ZINC, VNX & OBX) were tested in clean bedding, while the other half received intranasal infusions while being tested. Both the control and VNX groups had intact smell sense, while the ZINC and OBX groups were severely impaired.

Compared to testing in clean bedding, cat smell reduced pinning levels in the CON and ZINC groups by 81 and 30 percent, respectively, and rough-and-tumble activity by 64 and 15 percent. Play levels in the VNX and OBX were unaffected by the presence of cat odors. Cat odors also suppressed pinning activity and play levels in CON, ZINC and VNX groups, as it was increased in clean bedding during dissection of 4-6-10, apparently from the intact cat smell experience. Rates of play in the VNX2 and OBX groups remained stable.

Distribution of play among VNX group animals in the presence of cat smell indicates that the AHS may elaborate behavioral habit in the presence of olfactory cues which indicate danger to the animal.

444.6
THE PARAMETRIC STUDY OF A TASTE AVERSION MODEL OF DRUG DISCRIMINATION (DD) LEARNING. T. V. Jaeger* and E. P. Nau, Addiction Research Foundation, Toronto, M5S 2S1; and Dept. of Pharmacology, Univ. of Toronto, Canada.

At last year's meeting, data of Kautz et al. and Martin et al. suggested that taste aversion may provide a baseline for DD learning requiring high sensitivity, such as with naloxone in naive animals and with intraventricular infusions. Therefore, we manipulated and examined a model using SC drug to predict in thirsty rats (Sprague-Dawley males) whether or not an emetic follows presentation of a drug of palatable fluid. Differential consumption of the fluid was found during daily training with 0.04 mg/kg fentanyl or 20 mg/kg pentobarbital as the drug cue and 30 to 120 mg/kg LICI as the emetic, although learning was slower using two trials per day. As DD was seen whether the drug was presented in the absence of the aversion model, but the identification of parameters of high sensitivity may require more work.

([Supported by MA-9552-MRC and A-2000-NSERC]).

444.7
SOMATOSENSORY REGULATION OF NURSING BEHAVIOR IN RATS. P. S. Sacks and L. A. Johnson*. Psychology Dept., Rutgers University, New Brunswick, NJ 08803.

Based on pup retrieval ability, maternal behavior in rats is thought to be under multisensory control, but the sensory regulation of nursing behavior has been neglected. We assessed this using place changes at postnatal day 16/17. The results demonstrated that the somatosensory stimulation from pups. Dams were separated from their litters for 4-6 h prior to the cueing of test; pups were placed on a wet paper towel that was warm. Before nursing begins, dams typically engage in several oral behaviors, licking, and rearing over pups. Depivation of their nursing behavior in rats is thought to be under multisensory control, but the sensory regulation of nursing behavior has been neglected. We assessed this using place changes at postnatal day 16/17. The results demonstrated that the somatosensory stimulation from pups. Dams were separated from their litters for 4-6 h prior to the cueing of test; pups were placed on a wet paper towel that was warm. Before nursing begins, dams typically engage in several oral behaviors, licking, and rearing over pups. Depivation of their nursing behavior in rats is thought to be under multisensory control, but the sensory regulation of nursing behavior has been neglected. We assessed this using place changes at postnatal day 16/17. The results demonstrated that the somatosensory stimulation from pups. Dams were separated from their litters for 4-6 h prior to the cueing of test; pups were placed on a wet paper towel that was warm. Before nursing begins, dams typically engage in several oral behaviors, licking, and rearing over pups. Depivation of their nursing behavior in rats is thought to be under multisensory control, but the sensory regulation of nursing behavior has been neglected. We assessed this using place changes at postnatal day 16/17. The results demonstrated that the somatosensory stimulation from pups. Dams were separated from their litters for 4-6 h prior to the cueing of test; pups were placed on a wet paper towel that was warm. Before nursing begins, dams typically engage in several oral behaviors, licking, and rearing over pups.
444.9 **EFFECTS OF KAINIC ACID ON EMOTIONAL AND SENSOMOTOR BEHAVIOR IN DIPSYCHIC DOMESTIC CHICKS**. L. Normansell. D. Zeisloft* and J. Panksepp. Muskingum College, New Concord, OH 43762 and Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Intracarotid administration of kainic acid (K.A.), a conformationally-restricted analog of the excitotoxic amino acid glutamate, has been shown to induce vocalizations in chicks (Soc Neurosci Abstr 13: 763, 1987). This capacity of K.A to increase calling is more apparent when the chicks are tested under environmental conditions where baseline calling rates are generally reduced.

When chicks were tested alone surrounded by mirrors, in the dark in groups of four, when exposed to loud ambient sound levels, or when held in the cupped hands of the experimenter, KA-treated animals (1-25 µg) vocalized more frequently than did water-treated controls, suggesting that KA may disrupt processing of the visual, auditory, and somatosensory stimuli which influence vocal production.

Following KA administration, other behaviors of young chicks are also affected. KA-treated chicks fail to respond to a novel object placed into their immediate environment. They exhibit abnormal approach tendencies toward the remainder of their flock; they assume a fear-like squatting posture, and they make more errors than controls on a pebble floor task of visual discrimination.

Effects of KA on the current thresholds required to produce vocalizations by electrical stimulation were also investigated after stereotaxically-guided microinjection into the Midbrain Calling Region. Two min. after KA infusion (5 µg), stimulation thresholds were increased about 10X, whereas thresholds in control animals tended to decrease. Following surgery, KA-treated chicks showed a slight reduction in calling frequency when they were tested in isolation. Those animals, however, did not respond to the presence of their own mirror images by a suppression of calling, suggesting a long-term disruption of visual capability.

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Behaviors which follow each other in the behavior sequence by higher probability than chance tend to correlate positively, thus masking underlying motivational factors. A statistical method was developed to correct for the sequential effects on correlations among behaviors in the open-field (OF) test.

Of behavior of 200 mice, a complete generation of a long-term neurogenetic study, starting from F3 generations of 3 inbred strains (Vadass, C. et al, J. Neurogen., 4:241-252, 1978), was monitored using an event registration software, ETHOGRAM, developed in our laboratory. Occurrence of 16 behaviors was recorded. Behavior sequences were higher than second order Markov chains i.e. the probability of the occurrence of a behavior is dependent at least on the two preceding acts. Factor analysis of the corrected correlation matrix yielded 6 factors which were labeled as EXPLORATION (rearing and leaning), ESCAPE (climb and jump on wall), DIRECTION CHANGE (backstep while rearing and turn), URINATION and two GROOMING factors. Defecation had a negative loading on one of the grooming factors and a small positive one on "escape".

These results demonstrate that the narrow interpretation of OF tests (in terms of exploration and emotionality) is an oversimplification of the complexity of OF behavior.

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444.11 **SELECTED LINES OF RATS DIFFER IN THEIR RESPONSIVENESS TO SUCCESSIVE BLOCKS OF CONTRAST AND REVERSAL LEARNING PROCESSES**. G.A. ROWAN & C.F. FLAHERTY. Psychology Dept, Rutgers Univ. New Brunswick, N.J. 08904.

The consumption behavior of rats shifted from 32% to 4% sucrose declines to a level substantially below that of unshifted rats that have experienced only 4% sucrose. This negative contrast effect involves a stress component (corticosterone elevation) and the animal is attenuated by administration of the benzodiazepine tranquilizers.

The present experiments investigated the behavior of two selected lines of rats that have been shown to differ in "emotionality". The Maudsley Reactive (MR/Har) and Maudsley Nonreactive (MNRA/Har) rats and the Syracuse High and Low Avoidance rats (SHA/Bru and SLA/Bru) were both compared to a large population (N=397) of nonselected rats in terms of their negative contrast effect. The results demonstrated that the "nonemotional" rats in both selected lines (SHA and MNRA) showed reliably smaller decrements in licking when shifted from 32% to 4% sucrose than is typical in unselected rats. The SLA rats (high emotional) showed marginally larger contrast effects than the unselected rats. However, the MR rats, selected for open field defecation, showed significantly smaller contrast effects than the unselected population.

Chlordiazepoxide (8 mg/kg) was differentially effective in reducing the negative contrast in the selected lines.

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444.12 **EFFECTS OF ACUTE PHENYTOIN ON CORTICAL EVENT-RELATED POTENTIALS**. E.S. Baratz, S.A. Chappell, M.H. Brandt*. Dept. of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550-2777.

Acute dosages of phenytoin have been shown to have an effect on both the early and late cortical event-related potentials while subjects observed light flashes at three intensities (Barrett, et al., Neuropsychobiology, 15:201-207, 1986). In this double-blind, placebo controlled study, phenytoin (100mg) significantly decreased the amplitude of the N100 component (reducing) and enhanced the frontal negative potential of the slow wave but did not effect the posterior portion of the P300 wave form. The current study extended this research by: (1) using 2 dosages of phenytoin (100mg and 200mg); (2) having subjects perform 2 additional tasks (oddball and go/no-go). The phenytoin related regional differences in ERPs were consistent with the previous study in both dosages. However, the 200 mg dosage had more generalized cortical effects. These experiments involved the use of a topographical analysis of ERP's (Cappola, et al., Compr. Brain. Med. 12:191-192, 1982). The results are consistent with the hypothesis that phenytoin effects cognition by acting on frontal cortical areas during information processing.

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444.13 **UNIT ACTIVITY IN THE HIPPOCAMPUS AND TEMPORO-BASAL CORTEX RELATED TO ATTENTION AND MEMORY IN THE BEHAVING MONKEY**. T.R. Vidyasagar*, J.D. Kent and J. Feuston. Harvard Medical School, Boston, MA.

Single and multiunit activities were recorded from the hippocampus and temporobasal cortex, as the monkey (Macaca fascicularis) was performing a delayed matching-to-sample (DMS) task or during a variety of behaviour situations that involved attention, expectation, reward or position in exo- or endocentric space. Only a minority of neurones gave significant responses related to the DMS task. In contrast, many cells responded vigorously to situations involving the experimenter or particular emotional or motivational states of the animal. For example, many units responded (or were inhibited during the time) when the monkey was shown a piece of food that was about to be given to him or while consuming it. Others were activated or were extinguished when the experimenter entered or left the room, sometimes the response being dependent on the location of the event relative to the monkey. It is impossible to interpret the responses in the DMS tasks as being more related to changes in motivational or attentional states of the task than to the process of memory storage itself.

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444.14 **THE EFFECTS OF BENZODIAZEPINES, 5HT1A AGONISTS AND ETHANOL ON NATURAL ANXIETY/DEFENSIVE BEHAVIORS OF RATS TO A CAT**. R.J. Blanchard, D.C. Blanchard* and J. Rodgers. Psychology Department, Univ. of Hawaii, Honolulu, HI 96822.

Rates living in a Visible Burrow System connected to a "surface" area show a continuous activity; they were recorded from the hippocampus and temporobasal cortex, as the monkey (Macaca fascicularis) was performing a delayed matching-to-sample (DMS) task or during a variety of behaviour situations that involved attention, expectation, reward or position in exo- or endocentric space. Only a minority of neurones gave significant responses related to the DMS task. In contrast, many cells responded vigorously to situations involving the experimenter or particular emotional or motivational states of the animal. For example, many units responded (or were inhibited during the time) when the monkey was shown a piece of food that was about to be given to him or while consuming it. Others were activated or were extinguished when the experimenter entered or left the room, sometimes the response being dependent on the location of the event relative to the monkey. It is impossible to interpret the responses in the DMS tasks as being more related to changes in motivational or attentional states of the task than to the process of memory storage itself.
MOTIVATION AND EMOTION II

V. Hömberg * and H. Schuhmacher*, University of Düsseldorf

VISUAL STIMULI

We have shown before that several late positive components matematically relate to demand of semantic processing as well as to subjective experience of interest value. This study was designed to look at the influence of systematically related to subjective experience of interest value and stimulus repetition on ERPs to complex visual stimuli. 7 male volunteers were confronted up to 500 objects presented tachistoscopically for 150 ms. EEG was taken from various sites. Sectional electrode reference was used. Averages were composed over tasks of various complexity and subjective experience of interest value.

Principle component analyses revealed multiple partially overlapping late positive components: a parietally prepontented P300 and a fronto-centrally dominant P800 were systematically related to demand of semantic processing but not to interest value. 7 male volunteers were confronted up to 500 objects presented tachistoscopically for 150 ms. EEG was taken from various sites. Sectional electrode reference was used. Averages were composed over tasks of various complexity and subjective experience of interest value.

Multiple long latency ERP with different topography are systematically related to difficulty of semantic processing and subjective experience of interest value.

FEEDING AND DRINKING VII

OPIOID INJECTIONS INTO THE BED NUCLEUS OF THE STRIA TERMINALIS: EFFECTS ON FOOD INTAKE. B.A. Gossell, Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109-0116.

The stria terminalis is a major efferent pathway from the amygdala, and enkephalin-containing fibers have been demonstrated in this pathway and in the bed nucleus of the stria terminalis (BNST) (e.g., Uhl et al., Brain Res. 149:223-228, 1978). In this report, the possibility was tested that the BNST may contribute to the increased feeding observed after systemic or IC injections of opioid agonists. Cannulas were implanted unilaterally into the BNST of male Sprague-Dawley rats. In a repeated measures design, the mu opioid agonist DAGO was injected into the BNST at doses of 0 (NaCl), 0.1, 0.3, and 1 nmol. In a separate experiment, the selection of a delta agonist DTLET was tested at the same doses. The 1 nmol dose of DAGO caused a small but significant increase in 2 and 4 hr food intake. Intake was also increased after DTLET (1 nmol), although the effect fell just short of statistical significance. These experiments suggest that the BNST may contribute to the increased feeding observed after systemic or IC injections of opioid agonists.

The data suggest that changes in mu opioid system activity probably do not contribute to radiation induced 'appetite' suppression. Furthermore, the data suggest that, in clinical settings, pain control medications may not exacerbate radiotherapy induced appetite suppression.
FEEDING AND DRINKING VII

ANTIOXONE POTENTIATION OF EFFECTS OF CCK ON GASTRIC MOTILITY AND FEEDING. E. M. Stricker, E. M. Stricker, R. K. Stricker, and E. M. Stricker. Department of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Neurohypophyseal secretion of OT in response to dehydration, hypovolemia, and perturbation in rats is known to be mediated by the opioidergic antagonist naloxone (NX). The present studies demonstrated that stimulation of OT secretion by systemic injection of CCK (100 ng/kg) into male rats, body weight 300 g, increased 5 fold the OT secretion in the paraventricular area of the hypothalamus. This parallel potentiation by NX of CCK- and LClO injection-induced increases in OT secretion, gastric motility and food intake suggests that: 1) OT pathways are likely involved in some aspects of central control of feeding behavior, and 2) endogenous opioid peptides are likely involved in regulation of this and other oxytocinergic neural systems in rat brain.

Supported by National Research grant MH-25140, and training grant MH-18273-03.

OPPOSITE EFFECTS OF ROSTRAL AND CAUDAL VENTRICULAR INFUSION OF NON-BINDING ANTIBODY TO OXYTOCIN (OT) SECRETION IN LACTATING RATS. E. T. Biels, K. D. Carr, and K. D. Carr. Department of Psychology, NYU Medical Center, New York, NY 10016. and Department of Psychology, Christopher Newport College, Newport News, VA 23606.

The electrical brain stimulation threshold for eliciting feeding is elevated by centrally administered antibodies to the putative endogenous oxytocin receptor, antiserum (1-10 ug/kg, 100 ug, 400 ug) in the rat. However, these effects were not seen in male rats. In contrast, 1-10 ug/kg CCK increased the threshold for OT secretion in lactating female rats. The present study reports the opposite effects of rostral and caudal ventricular infusion of non-binding antibody to OT. THURSDAY PM

EFFECTS OF EXERCISE & RESTRICTED FEEDING ON WEIGHT LOSS AND BETA-ENDORPHIN LEVELS IN THE RAT: IMPLICATIONS FOR ANOREXIA NERVOSA. L. E. Doerries, P. F. Aravich, A. W. Nash, and B. Endorphin levels in the rat are affected by exercise and food intake. This is true for both basal and stimulated (agonist-driven) endorphin levels. In the present study, a similarly marked pattern was produced only by DYN [1-8] antibodies infused into the AQ. These results suggest that DYN [1-8] and [1-6] may both mediate SIF, and that DYN [1-8] activity in some caudal periventricular structures may contribute to the opioid mediation of feeding as revealed by naloxone antagonism. (Supported by NIDA grant DA03596 to K.D. Carr.)

EFFECTS OF VENTRICULAR INFUSION OF OXYTOCIN (OT) ON GASTRIC MOTILITY AND FEEDING. E. M. Stricker, E. M. Stricker, K. D. Carr, and K. D. Carr. Department of Psychology, NYU Medical Center, New York, NY 10016. and Department of Psychology, Christopher Newport College, Newport News, VA 23606.

Anorexia nervosa (AN) is associated with a variety of neuroendocrine, behavioral, and historical disorders, including an impaired ingestive response to 2-deoxy-3-glucose (2DG) and glucoprivation. Unfortunately, it is often difficult to distinguish between the primary abnormality of AN and the secondary consequence of severe emaciation. Because of increased interest in the relationship between exercise and AN, we are beginning a systematic examination of activity-based anorexia in the rat, which is produced by both groups of female rats: by 40-60% for 10-20 min after 10 ug/kg CCK, values comparable to those seen in male rats, but had no effect on OT secretion in lactating female rats. (Supported by NIMH research grant MH-25140.)


In our SIF paradigm, naloxone produces a marked pattern of progressive elevation in serially determined thresholds (Carr & Simon, Brain Res. 297:369, 1984). In the present study, a similarly marked pattern was produced only by DYN [1-8] antibodies infused into the AQ. These results suggest that DYN [1-8] and [1-6] may both mediate SIF, and that DYN [1-8] activity in some caudal periventricular structures may contribute to the opioid mediation of feeding as revealed by naloxone antagonism. (Supported by NIDA grant DA03596 to K.D. Carr.)

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EFFECTS OF VENTRICULAR INFUSION OF OXYTOCIN (OT) ON GASTRIC MOTILITY AND FEEDING. E. M. Stricker, E. M. Stricker, K. D. Carr, and K. D. Carr. Department of Psychology, NYU Medical Center, New York, NY 10016. and Department of Psychology, Christopher Newport College, Newport News, VA 23606.

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445.11
THE EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON FOOD INTAKE AND MATERNAL BEHAVIOR OF LACTATING AND POSTWEANING RATS.
S.A. Wager-Srdar and A.S. Levine.
Neuroendocrine Research Laboratory, VA Medical Center and University of Minnesota, Minneapolis, MN 55417.
Cholecystokinin octapeptide (CCK-8) decreases food intake in a number of species. Behavioral studies have shown that CCK-8 exerts no sequence of binding sites is similar to those of post-prandial satiety. It has been found that CCK-8's satiating effect is altered during lactation. We examined the effect of CCK-8 (5 µg/kg) IP on food consumption of lactating and postweaning (PW) rats. Food intake was determined by CCK-8 during the first and last week of lactation. It was found that food consumption was decreased by CCK-8 compared to the saline group (F(1,30) = 26.04). The maternal behaviors, nursing and pup interaction were not altered by CCK-8. However, the latency to the first meal was increased 33-47% during lactation but not during PW. The maternal behaviors, nursing and pup interaction were not altered by CCK-8. Maternal behavior was not affected by CCK-8 in this study.

445.12
Streptozotocin-induced diabetes in rats results in development of hyperphagia and changes in hormone pancreatic and gastrointestinal function. The following study was carried out to determine the relative sensiti- vity of DB and control (C) rats to satiety induced by CCK-8 administered IP, and to determine changes in sensitivity over time after induction of diabetes. Male Sprague-Dawley rats (6/group) were given 0.2 and 4 µg/kg CCK-8 IP (Latin square design) after 6 hr fast on weeks 1, 3, 5, and 9 after administration of streptozotocin (DB) or control (C). As early as 1 wk after DB rats failed to respond to CCK-8, whereas there was a clear dose-response effect in C rats. By week 9 there was still no significant decrease in food intake for DB rats, although there was trend toward a decrease. C rats still exhibited a significant dose-related decrease in intake. Diabetes results in increased suppression of feeding induced by a function partially controlled by CCK induced contraction of the pyloric sphincter. The slowing of emptying results in satiety, in which may be an important signal of satiety. Defects in CCK receptor mechanisms have been identified in the pancreas of DB rats; the same is true for pyloric CCK receptors. This study was supported by NIH NS20000 and Monsanto Co.

445.13
ROLES FOR BOTH PYLORIC AND VAGAL CCK RECEPTORS IN THE REGULATION OF FEEDING AND DIETARY SENSITIVITY. G.C. Blatter. Dept. of VCAPP, Washington State University, Pullman, WA 99164.
The suppression of food intake after peripheral administration of bombesin (BBS) is partially mediated by spinal-visceral afferent neurons (Stuckey et al., 1985). High dorsal column transection interrupts a population of neurons which participate in the satiety system in BBS-induced suppression of feeding. We have found that the suppression of feeding induced by a peripherally administered BBS was significantly attenuated in rats with dorsal column transection compared to sham-operated controls (p < .02). Intraplantar injection of 4 µg/kg BBS reduced 30 min intake of a palatable solid food (cookies) by 63.1% ± 5.8 in control rats while those with dorsal column transection reduced intake by 41.6% ± 7.0. Food intake following a baseline injection of 0.5% saline was not significantly different between groups (p > .05). These results indicate that high spinal cord transection of the dorsal column pathway interrupts a population of neurons which participate in BBS-induced suppression of feeding.

446.1
Pharmacological treatment of opioid addicts have used either methadone (MET), a partial agonist, or naltrexone (NLX), an opiate antagonist. Successful detoxification of MET to NLX has been identified in the clinic. Buprenorphine (BUP) is a partial agonist which at higher doses can act as an opioid antagonist. An optimal treatment dose would substitute for MET without withdrawal symptoms, block opioid induced euphoria, and then allow detoxification to NLX. To determine this dose of BUP, we assigned 39 opiate addicted patients to one of 4, 8, or 4 µg/kg BUP dose or control. Three out of 12 patients show signs of partial antagonist activity. Patterns of withdrawal symptoms differed across groups with the 4 µg group showing a less typical withdrawal symptom profile. The significant effects of time (p < .01) and dose x time (p < .05). A moderate dose of BUP shows promise as a transitional agent from MET maintenance to NAL abstinence without causing serious withdrawal symptoms.

446.2
L-HISTIDINE BLOCKS THE EFFECTS OF PENTAZOCINE ON BRAIN-STIMULATION REWARD. S. Rasnokick, C. Decker*, and C. Korneetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.
Previous results indicate that the antihistaminic tripelennamine potentiates the threshold lowering effects of pentazocine (PEN) (Farn. Biochem. Behav. 81:261, 1984) on brain-stimulation reward, a model of drug-induced euphoria. To determine histamine's role in this interaction we studied the effects of the combination of L- histidine and PEN on the threshold for brain-stimulation reward. As in previous studies PEN alone (2.5-10.0 mg/kg) lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold. A combination of 100 mg/kg PEN and 1000 mg/kg L-histidine lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold. A combination of 100 mg/kg PEN and 1000 mg/kg L-histidine lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold. A combination of 100 mg/kg PEN and 1000 mg/kg L-histidine lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold. A combination of 100 mg/kg PEN and 1000 mg/kg L-histidine lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold. A combination of 100 mg/kg PEN and 1000 mg/kg L-histidine lowered the threshold for reward stimulation to the median solution in rats but L-histidine (250 and 500 mg/kg) did not alter the threshold.
446.3 PRECIPITATED WITHDRAWAL IN MONKEYS AFTER REPEATED DAILY ADMINISTRATION OF DIFFERENT BENZODIAZEPINES.


Relative physical dependence potential of four marketed benzodiazepines was assessed in monkeys in a flumazenil-precipitated withdrawal paradigm after the repeated oral administration of doses previously shown to be equieffective in producing a loss of righting reflex.

Squirrel monkeys received 18 daily oral administrations of 2 mg/kg alprazolam (A; N=4), 30 mg/kg diazepam (D; N=4), 1 mg/kg flunitrazepam (F; N=4), 280 mg/kg oxazepam (O; N=5) or vehicle (V). A second flumazenil injection 5 hours after the ninth daily treatment precipitated withdrawal (convulsions, tremor and/or vomiting were observed during a subsequent 1 hour period) in 4 A-, 4 D-, 3 F-, 4 O-, and no vehicle-treated monkeys. A second IV flumazenil injection given 5 hours after the eighteenth daily treatment resulted in withdrawal in 4 A-, 4 D-, 3 F-, 3 O-, and no vehicle-treated monkeys.

Thus, repeated oral administration of A, D, F, or O at equieffective doses produced physical dependence which was manifested as flumazenil-induced withdrawal signs in the majority of monkeys.

446.5 STIMULUS PROFILES OF AGONIST-ANTAGONIST OPIOIDS IN A DISCRIMINATION TASK USING TWO DOSSES OF SALINE, ETHANOL, AND MORPHINE IN PIGEONS.

A.M. Young, N.A. Walton, and A.A. Perkins. Dept. of Psychology, Wayne State University, Detroit, MI 48202.

Experiments evaluated the discriminative stimulus profiles of selected opioids in pigeons trained to discriminate among saline and two doses of morphine (MS). Pigeons (N=15) were trained to discriminate among 1m. injections of saline (S), 1 mg/kg MS (the low dose or LD cue), and 10 mg/kg MS (the high dose or HD cue). Performance was maintained under FR 20 schedules of food delivery. After establishment of stimulus control, various doses of MS, alone and in combination with naloxone, and doses of other opioids were tested for generalization. For MS itself, LD generalization predominated at doses of 1.0 to 3.2 mg/kg; HD generalization at doses of 5.6 to 22 mg/kg. Naltrexone pA2 values for antagonism of the LD and HD cues were similar, suggesting that the discrimination was based on quantitative differences between the LD and HD cues. The agonists etorphine and methadone, and the agonist-antagonists nalbuphine and nalorphine evoked generalization to only the LD cue and, in other experiments antagonized the HD cue. These profiles suggest that a discrimination among saline and different MS doses may provide information about the agonist efficacy of agonist-antagonist opioids. (Supported by DA03796.)

446.6 EFFECTS OF OPIATE AND DOPAMINERGIC DRUGS ON NOVELTY PREFERENCE BEHAVIOR. M. L. Bardo, F. L. Herkenham, and R. C. Pieri*. Dept. of Psychology, University of Kentucky, Lexington Kentucky, 40506.

Previous research has demonstrated that animals prefer a novel environment over a familiar environment. The present studies investigated the role of opioid and dopaminergic systems in novelty preference behavior. Rats were familiarized with a distinct environment by being confined to that environment for 30 min per day on eight consecutive days. On the ninth day, rats received either morphine sulfate (0.0, 0.1, 0.3, 1.0 or 3 mg/kg, s.c.), naloxone hydrochloride (0.0, 0.1, 0.3 or 1.0 mg/kg, s.c.), amphetamine sulfate (0.0, 0.1, 0.3 or 1.0 mg/kg, s.c.) or haloperidol (0.0, 0.3, 0.1, 0.5 or 1.0 mg/kg, i.p.). Thirty min after injection, rats were allowed free-choice access to the familiar and novel environment for a novel environment for 15 min.

As expected, saline-injected control animals spent significantly more time in the novel environment than in the familiar environment. More important, the novelty preference behavior was counteracted in a dose-dependent manner by haloperidol, whereas amphetamine, morphine and naloxone had no significant effect. These results indicate that novelty preference behavior involves a dopaminergic substrate.

(Supported by USPHS grant DA 05312.)

446.7 NALOXONE-INDUCED REDUCTION OF VOLUNTARY ETHANOL INTAKE CORRELATES WITH SPONTANEOUS PREFERENCE.


Recently, the possibility of a common mechanism for the reinforcing properties of ethanol and opiates has been proposed on the basis of biochemical and behavioral data. This prompted us to study the effect of oral administration of doses previously shown to be equieffective in producing a loss of righting reflex. In squirrel monkeys received 18 daily oral administrations of 2 mg/kg alprazolam (A; N=4), 30 mg/kg diazepam (D; N=4), 1 mg/kg flunitrazepam (F; N=4), 280 mg/kg oxazepam (O; N=5) or vehicle (V). A second flumazenil injection 5 hours after the ninth daily treatment precipitated withdrawal (convulsions, tremor and/or vomiting were observed during a subsequent 1 hour period) in 4 A-, 4 D-, 3 F-, 4 O-, and no vehicle-treated monkeys. A second IV flumazenil injection given 5 hours after the eighteenth daily treatment resulted in withdrawal in 4 A-, 4 D-, 3 F-, 3 O-, and no vehicle-treated monkeys. A second IV flumazenil injection given 5 hours after the eighteenth daily treatment resulted in withdrawal in 4 A-, 4 D-, 3 F-, 4 O-, and no vehicle-treated monkeys. A second IV flumazenil injection given 5 hours after the eighteenth daily treatment resulted in withdrawal in 4 A-, 4 D-, 3 F-, 4 O-, and no vehicle-treated monkeys.

Thus, repeated oral administration of A, D, F, or O at equieffective doses produced physical dependence which was manifested as flumazenil-induced withdrawal signs in the majority of monkeys.

446.8 THE EFFECTS OF PRENATAL, POSTNATAL AND COMBINED EXPOSURE TO ETHANOL ON LEARNING AND ON HIPPOCAMPAL CELL DENSITY IN WELLMING RATS. T. Wiga, N.J. Lobaugh and A. Amel. University of Texas, Austin, TX 78712.

Neonatal rats were exposed to ethanol during postnatal Days 4 to 12 by employing an artificial rearing procedure with rats that orally self-administered ethanol. Bipolar stainless steel electrodes were stereoanatomically implanted in the medial forebrain bundle of male F-344 rats. Animals were water deprived, and then allowed to drink an ethanol-water solution prior to testing. Significant threshold lowering effects were observed, which persisted throughout the testing session, at doses between 0.8 g/kg and 1.6 g/kg depending on the individual animal's sensitivity. These threshold lowering effects in animals self-administering ethanol suggest that the same pathways that mediate the reinforcing effects of other abused substances may be involved, at least in part, in the reinforcing effects of ethanol. [(Supported in part by NIAAA grant DA09590 and Research Scientist Award DA 00099 (CA)].
**446.9**

BITHALIC EFFECT OF THC ON GLUCOSE UPTAKE IN RAT HIPPOCAMPUS. J. E. Margulies*, M. Hammer, Dept. of Anatomy & Reproduc­
tive Biology, Univ. of Hawaii, Honolulu, HI 96822.

Delta-9-tetrahydrocannabinol (THC) causes memory impairment and neurophysiologic alteration in hippocampus. We used the titrated 2-deoxy-D-glucose (2DG) autoradiographic method to examine the effect of acute administration of THC on regional brain metabolism in the hippocampus of male rats. Various doses of THC (0.2, 0.5, 2.0 and 10 mg/kg), solubilized in 5% propylene glycol and 0.5% Tween 80, were injected via intracardiac cannulae followed in 10 minutes by a pulse of 2DG (1.0 mCi/250 g). Animals were decapitated forty-five minutes after 2DG ad­ministration. Sham-operated control animals received the same dose of vehicle without THC. THC altered 2DG uptake in a biphasic manner in hippocampus and other limbic structures. The 0.2 mg/kg dose significantly increased 2DG uptake in the stratum lacunosum-moleculare (SLM) of the hippocampus, whereas doses of 2.0 and 10 mg/kg significantly decreased 2DG uptake in the SLM. The produced differential effects on the pyramidal cell layer and stratum oriens of CA1 and CA2-3. These layers in CA2-3 responded to THC biphasically, however 2DG uptake in these regions of CA1 was unaltered at low doses of THC. Thus, certain regions of the hippocampus are more sensitive to THC. This dose sensitivity may reflect a specific effect of THC as opposed to a nonspecific effect seen at higher doses. Supported by USPHS awards ROI DA03985 and K04 NS01161.

**446.10**

Á-1-TETRAHYDROCANNABINOL (THC) INHIBITS ARACHIDONIC ACID ACYLTRANSFERASE (AACAT) IN GUINEA PIG CEREBRAL CORTEX SLICES. M. Reichman*, N. W. Nen*, E. R. Hamer, Jr., Dept. of Pharmacology, Univ. of Wisconsin Med. School, Madison, WI 53706

The mechanism of action of THC, the major active ingredient in marijuana, remains unclear. We have found that THC increases unesterified arachidonic acid (AA) levels in guinea pig cerebral cortex slices prelabeled with [14C]AA. We report here that the mechanism underlying this rise involves an inhibition of AA acylation, rather than activation of lipolytic enzymes. The incorporation of AA into brain lipids was measured by incubating cerebral cortex slices with [3H] AA for 1 hr at 37°C without and with THC. The lipids in the tissue were extracted and separated by thin layer chromatography, and the radioactivity in the individual phospholipids and neutral lipids was measured. Treatment with THC concentrations in the range of 2-32 µM elicited dose-dependent and saturable reductions in the unesterified [3H]AA levels in membrane lipids. The IC50 was on the order of 8 µM, and a maximal reduction in radioactivity of 50% occurred at 32 µM. We observed concomitant rises in the levels of unesterified [3H]AA. The levels of radioactivity were significantly reduced in both the neutral lipid and phospholipid fractions, although the largest decreases in radioactivity were observed in phosphatidylinositol; the levels of [3H]AA in phosphatidylcholine were not significantly affected by THC. Our results indicate that the mechanisms underlying the THC-induced elevation in unesterified AA involve an inhibition in the acylation of membrane lipids, and suggest that insolubilizing phospholipids may play an important role in the mechanisms mediating the response.

Supported by ADAMHA Grant DA05699 and by NRSA Fellowship DA05307.

**447.1**


Intracellular recording and horseradish peroxidase injection techniques were used to characterize 84 neurons from the stratum griseum superficiale and stratum opticum of 42 hamsters that sustained removal of both eyes on the day of birth. Of the 84 recovered neurons, 90.5% (76) responded to somatosensory stimulation. These responses were often weaker and receptive fields were more diffuse than for somatosensory neurons recorded in the deep laminae. The vast majority (65.2%) of the recovered neurons were horizontal cells; 15.2% were widefield vertical cells, 13.2% were narrow field vertical cells, 6.3% were stellate cells, and the remainder (15.6%) could not be classified. In the superficial SC laminae of normal adult hamsters (Mooney, R.E. et al. J. Neurosci. 5:2989, 1985), horizontal cells constituted only 13.6% of a sample 59 recovered neurons; 20.3% were widefield vertical cells, 17.0% were narrow field vertical cells, 23.7% were stellate cells, 13.6% were marginal cells, and 11.9% could not be classified. This suggests that the change in afferent input to the superficial laminae that follows neonatal enucleation may result in either dendritic reorganization, or even the complete loss of neurons with horizontally oriented dendrites. Supported by ET 04170 and BNS 85 00142.

**447.2**


Immunohistochemical labeling with an antibody to GFAP (Boehringer), on the other hand, reveals little staining within the midline. The major exception to this is the group of squaerial cells which are intensely GFAP positive and observed on postnatal days 1-3 (P1-3, where P=day of birth); it is considerably decreased on P5-7. In the horizontal and vertical fibers, staining is also observed on P5-7. A group of labeled cells around the aqueduct of Sylvius shows a tuft of processes that are positioned along the folium and feet and portions of radial cell processes which are intensely GFAP positive. A group of labeled cells which are intensely GFAP positive are also observed at the surface persisting at least until P7. A group of labeled cells around the aqueduct of Sylvius shows a tuft of processes that are positioned along the folium and feet and portions of radial cell processes which are intensely GFAP positive. A group of labeled cells which are intensely GFAP positive are also observed at the surface persisting at least until P7. Support: NIH grants EY02621, T32GM07484.

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448.1


Immunohistochemical labeling with an antibody to GFAP (Boehringer), on the other hand, reveals little staining within the midline. The major exception to this is the group of squaerial cells which are intensely GFAP positive and observed on postnatal days 1-3 (P1-3, where P=day of birth); it is considerably decreased on P5-7. In the horizontal and vertical fibers, staining is also observed on P5-7. A group of labeled cells around the aqueduct of Sylvius shows a tuft of processes that are positioned along the folium and feet and portions of radial cell processes which are intensely GFAP positive. A group of labeled cells which are intensely GFAP positive are also observed at the surface persisting at least until P7. Support: NIH grants EY02621, T32GM07484.

447.3 EARLY DEPRIVATION PRODUCES MORPHOLOGICAL AND MORPHOMETRICAL CHANGES IN CAT LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX. A. G. Gutierrez and S. Hoff, School of Neuroanatomy, Yale Univ. New Haven, CT 06510.

Neurons that represent a surface area of the retina are normally restricted to layers IV and VI of the LGN. In dark-reared cats, the number of antibody positive cells is reduced. The loss of expression of an antigen (or antigens) recognized by Cat-304 and Cat-301 is mediated by visual experience from birth.

Supported by CNPq Brazilian Fellowship to A. G. and by NEI.

447.4 POSTNATAL DEVELOPMENT OF NEURONS AND OPTIC TRACT AXON ARBORS IN THE HAMSTER DORSAL LATERAL GENICULATE NUCLEUS: IN VITRO HRP STUDY. G.E. Shope 1,2, R.L. Lieberman 2, and D.O. Frost 1. 1Dept. of Neuroanatomy, Yale Univ., New Haven, CT 06510 and 2Dept. of Anatomy, Univ. Coll. London, WC1E 6BT.

Neurons and optic tract (OT) axons (including terminals) in the dorsal lateral geniculate nucleus (LGN) were labeled with HRP in brain slices and their morphology analyzed. Ommatidial axons project to LGN from the entire retina of the hamster and form arbors at these ages, most class 2 neurons do not. Dorsal nucleus of the lateral geniculate body (LGN) was studied. A few axons cross the LGN, most are in a columnar pattern in the LGN. In the LGN, most class 2 neurons do not form arbors at these ages, most class 2 neurons do not. Dorsal nucleus of the lateral geniculate body (LGN) was studied. A few axons cross the LGN, most are in a columnar pattern in the LGN.


The distribution of morphologically distinct retinogeniculate terminals was analyzed in the LGN of normal and retinectomized hamsters which had one eye removed on postnatal day 1. Retinal terminals were labeled with HRP implants in the optic tract (OT) ipsilateral or contralateral to the remaining eye. Normally there is a persistence of OT terminals within the dorsal nucleus of the lateral geniculate body (LGBd) (see Jhaveri et al., Neurosci. Lett. 46:287, 1984). In retinectomized hamsters, the LGBd and an expansion of the territories occupied by the remaining eye. Contralaterally, the unilaterally projecting terminal zone is occupied by OT terminals only. Unilateral OT terminals are also present in the superficial zone, where they normally would not be present. Furthermore, OT terminals, normally located superficially, now form abnormally large clusters and extend deeper into the nucleus. Ipsilaterally, to the remaining eye, the expanded retinogeniculate terminal is composed of OT and OT terminals. In these cases OT terminals are seen subjacent to the OT, a location normally occupied by OT terminals from the contralateral eye. OT terminals, on the other hand, are not present in the expanded ipsilateral projection.

These results indicate that morphology of retinogeniculate axon terminals may be dictated by retinal ganglion cell specificity and survival rather than by their target environment. The altered distribution of different terminal types within the LGN, following neonatal monoclonal enucleation, also suggests an anomalous convergence of inputs from different types of retinal axons onto geniculate neurons.


Lesions of the superior colliculus (SC) in neonatal hamsters are followed by the appearance of abnormal retinal ganglion cell axons that project from the SC to the lateral posterior nucleus (LP). Immunocytochemical localization of GAP-43, a putative regulator of synaptogenesis, was not reduced. In the SC, anti-GAP-43 staining was excluded from the areas of the LP that received retinal input. In the areas of the LP that receive retinal input, the anti-GAP-43 staining was not reduced. This result suggests that GAP-43 may play a role in the development and maintenance of synaptic connections between retinal ganglion cells and their target neurons in the LP.

Supported by NSF, NIMHD.


In these experiments, we examined how the adult structure of geniculo-cortical neurons develops by injecting rhodamine microspheres into the visual cortex of early postnatal and adult cats and, subsequently, viewing brain slices of the LGd with epi-fluorescent microscopy so that their morphology could be related intracellularly with FITC-HRP. In the adult brain slice, all three classes (1, 2, and 4) of geniculate projection neurons were recognized. In the neonate (Kittens 3-5 weeks of age), neurons resembling each morphological class also could be identified. Although many class 1 and class 2 neurons could be matched intracellularly with the visual field possession adult-like dendritic arbors at these ages, most class 2 neurons do not. Despite these morphological similarities, geniculate projection neurons could be matched intracellularly with the visual field possession adult-like dendritic arbors at these ages, most class 2 neurons do not. Despite these morphological similarities, geniculate projection neurons could be matched intracellularly with the visual field possession adult-like dendritic arbors at these ages. These include more proximal dendrites, an increase in the number and assortment of dendritic appendages and, occasionally, exuberant intracortical axonal arbors. These transitory dendritic features are not seen in the adult.

Supported by EY06591, NSF8519709, MIGH9583, and NSF8606570.

447.8 ANALYSIS OF SYNAPTOGENESIS USING MULTIPLE ULTRASTRUCTURAL CRITERIA IN THE RAT VISUAL CORTEX AND SUPERIOR COLLICULUS. B.N. Bakum, J. Benavente, and R. Cohen. Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, IL 60612.

We examined the developmental patterns and time course of synaptogenesis in the developing rat visual cortex (VC) and superior colliculus (SC) using electron microscopy. The parameters measured have already been implicated as morphological correlates of plasticity and have been extensively used to determine the early stages of development. In the VC, synapses were found in the early stages of development. In the SC, synaptogenesis was observed beginning at P0 and reaching a peak at P14, after which synapse number declined. In the VC, synapse number at P0 was similar to that at P14, whereas synapse number at P0 was similar to that at P14, whereas synapse number at P0 was similar to that at P14, whereas synapse number at P0 was similar to that at P14.

Supported by NIH Grant NS 15889.
EFFECTS OF VISUAL DEPRIVATION ON DENDRITIC MATURATION OF STRIATE CORTEX. T. C. Trusk, M. Wong-Riley, and W. Kaboord. 2 Dept. of Anatomy, Creighton University, Omaha, NE 68178 & Dept. Physiology, Medical College of Virginia, Richmond, VA 23298. 447.12

To study the maturation of the projections from the medial bank of lateral suprasylvian (LS) sulcus to superior colliculus (SC), fast blue (FB; a long-lasting tracer) was injected in the SC at one day postnatal (dpn). At 27 dpn a nuclear yellow (NY) injection was made that encompassed the fiber tracts that projected to SC at birth (i.e. FB labeled) were present homogeneously throughout layer V. Far fewer neurons maintained their projections at 27 dpn (i.e. double-labeled) but these had the same distribution as the FB-labeled neurons. Neurons labeled only with NY were rare. The possibility that corticocortical projections had 'inappropriate' targets in SC was also evaluated. Injections of tritiated leucine were made in the medial bank of LS at 0 and 14 dpn. The terminal labeling pattern indicated that at birth, as in adulthood, the medial bank terminates topographically in the superficial laminae and dorsal aspect of the internuncial laminae of the ipsilateral SC. Therefore, although corticocortical connections from medial suprasylvian cortex are lost during maturation, their loss (like those from striate cortex) reflects refinements within the mature target zone in the SC.

Supported by Health Future Found. & NIH grant EY05626.

EFFECT OF MONOCULAR LID SUTURE, ENUCLEATION, AND RETINAL IMPULSE BLOCKAGE ON CYTOCHROME OXYDASE ACTIVITY IN LAMINATIONS OF MACAQUE STRIATE CORTEX. C. G. Truss, M. Wong-Riley, and W. Kaboord. 2 Dept. of Anatomy & Cellular Biology, Med. Coll. of WI, Milwaukee, WI 53226.

We sought to quantify the size and number of cytochrome oxidase-reactive and nonreactive neurons in control and experimental supragranular puffs (CP & EP) and intra control and experimental interlaminar (IC) & IEI monkeys subjected to monocular lid suture (MD), monocular enucleation (ME), or retinal impulse blockage with tetrodotoxin (TTX). Numbat anesthesia (30 mg/kg) was used during surgery and euthanasia. We found no cell loss in all experimental monkeys. The average size of reactive neurons in CP and ICI were similar to those of normal monkeys. Numerical densities of reactive neurons were decreased in adenocortical monkeys. Their average size was reduced in MD and TTX. However, in a long term ME, the sparse population of remaining reactive cells were mainly medium and large in size. The average size of nonreactive neurons in EP remained similar to that found in CP. In contrast, they were consistently smaller in ICI than in IC, and this difference was significant in adult and juvenile MD’s (11 to 48 weeks). Optical densities (CD) of EP and ICI were consistently lower than those of CP and ICI. This depression was less severe in adult monkeys lid sutured for 11 weeks or given intraretinal TTX for 2 weeks; and was most severe in an adult treated with TTX for 4 weeks. Optical density of CP was within the range found in normal monkeys, while OD in ICI was consistently greater, and OD in ICI was lower than normal interlaminar. These results suggest that: a) metabolically active neurons of lamina II-III are more sensitive than less active cells to sensory deprivation in adults; and b) there is an increase in metabolic activity within the interpuff region innervated by spared eye. [Supported by NIH EY07016 (TCT) and EY05459 (MWR)].

The organization of cells which comprise the projection of the corpus callosum (CC) of the rabbit are exuberant at birth and normally regress postnatally. We have examined the development of the 17/18 border in adults. Monocular enucleation (ME) on the day of birth results in an exuberant CC cell distribution in the adult similar to that observed in the neonate. Dark-rearing results in a CC cell distribution which is more sparse than normal and which extends into the medial portion of area 17 further than in the normal adult rabbit but not as far as in the neonate or the ME rabbit. In the present study we examined the effects of neonatal binocular enucleation (BE) on the CC cell distribution. Seven Dutch-Belted rabbits had BE on the day of birth. After reaching adulthood, multiple injections of HRP (Sigma Vl. 20% in H2O2) were made throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut in 1 µm sections with TMB. BE rabbits had a sparse CC cell distribution similar to that observed after dark-rearing. However, the CC cell distribution was not exuberant. In addition, there were fewer cells in the infragranular layers of BE rabbit compared to either normal, ME, or dark-reared rabbits. The results of the present study provide further evidence that visual experience and the presence of the primary afferent visual pathway make different contributions to the postnatal development of the corpus callosum. Supported by NIH grants EY06996 and EY02488.

VISUAL CORTEX TRANSPLANTED TO FRONTAL REGION FORMS RECIPROCAL THALAMIC CONNECTIONS TYPICAL OF MOTOR CORTEX. Dennis D.M. O'Leary, Dept. of Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

In an attempt to define further the influence that the position of a developing cortical neuron in the tangential plane of the visual field has on the connections that it establish, pieces of E17 fetal occipital cortex, exposed to 3H-thymidine in utero on El6, were transplanted to the frontal region of newborn rats. The host rats were allowed to mature (P5 to P 20) and HRP was then iontophoresed into the transplant. 1-2 days later, the rats were perfused and their brains examined. These allometric measurements of eye and brain size. These allometric measurements were compared to those cells, and longer gestation and maturation. In this study we have investigated the relationship between neurogenesis and allometric measurements of eye, brain and body size. These allometric measurements were compared to those of normal adult rabbit. The results of the present study provide further evidence that visual experience and the presence of the primary afferent visual pathway make different contributions to the postnatal development of the corpus callosum. Supported by NIH grants R01 NS1245 and KO4 NS00783.

TROPHIC AGENTS VI


Although not requiring nerve growth factor (NGF) for survival (RYM, J. Neuroscience, in press, 1986), adult rod dorsal root ganglion (DRG) neurons cultured with NGF show elevated levels of a number of endogenous neuropeptides. Substance P (SP) and calbindin gene-related peptide (CGRP) (Lindsay et al., 1987, Neuroni. & Vet. 31, 518). To confirm that the effect of NGF is due to an increase in the rate of synthesis of these two peptides, we have studied the effect of NGF on the rate of synthesis of these peptides. After culture for periods between 4-8 days with or without NGF, total cellular RNA was isolated by incubation of adult rat DRG cultures in 20 ml of 10% guanidinium HCl followed by phenol/chloroform extraction. Messenger RNAs coding for the peptides SP (preproSP) and CGRP (preproCGRP) were detected by isopycnic centrifugation of the RNA on 5-20% formaldehyde gels followed by autoradiography. In cultures deprived of NGF, both SP and CGRP mRNA levels declined with time in culture. In NGF-treated cultures, increased levels of both mRNAs were detectable within 24h and after 5 days levels of SP and CGRP mRNA were over 20-fold higher than in control cultures. Even when cultures were deprived of NGF for 5 days, similar increases in the levels of both mRNAs were achieved upon readdition of NGF indicating, that the effects of NGF are not mediated through enhanced survival of PPT and CGRP-producing neurons. We suggest that NGF may exert a continuous trophic influence upon mature sensory neurons through regulation of specific neuronal functions such as the expression of certain neuropeptides, as shown here.
448.3 EARLY GENE REGULATION BY NERVE GROWTH FACTOR: INDUCTION OF AN ADENYLATE CYCLASE-LIKE GENE, C. E. Cipollone, Department of Neurobiology, Stanford University, School of Medicine, Stanford, CA, 94305.

Nerve growth factor (NGF) is a neurotrophic molecule responsible for the maintenance of sympathetic neurons, other sensory neurons, and some adult mammalian neurons. NGF also induces chromaffin cells, a neural crest-derived tissue, to differentiate into sympathetic nervous system (SNS) cells. In order to gain some insight into the early steps of this differentiation process, we made a DNA library from PC12 cells (a line derived from a chromaffin cell tumor) one hour after treatment with NGF. We identified two clones (Tirone, F., and Shooter, E.M. Soc.Neurosci.Abstr.13(1):SS1,1987), PC1, PC2, PC3, and PC4, corresponding to different mRNA species highly induced by NGF as well as by epidermal growth factor (EGF) and cyclic AMP. Amino acid sequence analysis showed that PC1 corresponds to NGF-A, recently described as an NGF-inducible cDNA encoding for a transcriptional regulatory factor, using as a partial sequence of a putative mouse β interferon (Skup, D., et al. Nucl.Acids.Res.10,3069,1982). We cloned a full-length cDNA copy of PC1 and found that its deduced protein sequence, 449 amino acids, was significantly related to the sequence of the rat interferon protein. The expression of mRNA in tissue showed that PC1, unlike the other mRNAs, is present in the adult rat brain, beginning as early as 7 days after birth, in a period related to growth and differentiation of the glial cells. PC2, PC3 and PC4 mRNAs are instead expressed in placenta and tissues rapidly proliferating and differentiating - and in the neural tube (with the exception of PC2) between 12 and 14 days of gestation, in a period related to neurulation and, in part, differentiation. An increase (for PC1) or a decrease (for PC4) of expression was also observed in neoplastic cell lines in a differentiative and/or proliferative events, triggered by NGF or by other factors in the brain or in non-neuronal tissues. PC4 could exert this action, in analogy to that of interferon and lymphokines (regulators of cell proliferation and differentiation) by influencing gene expression at levels (e.g. RNA stability) other than RNA transcription (as could be for PC1).

448.5 DEVELOPMENTAL EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGF-R) mRNA IN HYPERTENSIVE AND NORMOTENSIVE RATS, K.A. Curran, R.J. Riopelle, R.J. Heddle, (SPOK: C.R. Craig) Dept. of Pharmacology & Toxicology, West Virginia University, Morgantown, WV 26506.

NGF receptors were measured in order to examine the role of nerve growth factor receptor gene expression in the etiology of hypertensive encephalopathy in the genetically spontaneously hypertensive rat (SHR). Total RNA (h), sorta (A) and mesenteric artery (M) RNA was extracted from 10, 60 and 100 d SHR and normotensive Wistar Kyoto (WKY) and hybridized with NGF-R riboprobes. The degree of sympathetic innervation in the adult rat is A > H > M. Expression of NGF-R was developmentally regulated in all 3 tissues examined. Levels in M and A were 10-fold greater in 10 d than in the 60-100 d rats. Highest NGF-R levels in 10 d rats were seen in H and fell to 3-fold at 60-100 d. Levels of NGF-R mRNA in M and A of both rat strains, and in WKY and the in 10 d rats. The NGF-R content in 10 d SHR M and H times those in WKY rats, but was not statistically significant.

Thus, NGF-R mRNA levels at the ages tested do not correlate with the degree of sympathetic innervation seen in the adult. Independent assays from this laboratory showed that NGF mRNA in M is 5 times greater in the 10 d SHR than WKY. Hyperinnervation of SHR M therefore correlates with elevated expression of NGF, but not NGF-R gene expression. (Supported by NIH grant HL 36885).

448.6 DISTRIBUTION OF PRO-NERVE GROWTH FACTOR-LIKE IMMUNOREACTIVITY (PRO-NGF-LI) IN THE ADULT RAT BRAIN, M.C. ZIEGLER, E. DIOQUA, Y. LAMOUX and F. BRACKET (SPOK: S. DROUVA) D161, 2, rue d'Alesia 75014 PARIS and INSEER, 6802, CHB, 69032 ARBOIS, FRANCE.

In the adult rat, the localization of the pro-NGF has been studied in various brain and spinal cord regions, using affinity-purified sera raised against three synthetic peptides that reduce epitopes of the pro-NGF, and immunohistochemical (IHC) methods. The three antibodies labeled similar regions, but each sera resulted, for a given structure, in a specific distribution pattern. Strong pro-NGF-LI is present in neocortex, mediaspinal cord, hippocampus, globus pallidus, some thalamic nuclei, olfactory bulb, reticular nuclei of the brain stem, whereas lateral septum, hypothalamic nuclei, cerebellum, substantia nigra, motorneurons of the ventral horns, the intermediolateral columns and dorsal horns showed less intense labeling. The pro-NGF-LI was mainly observed within cell bodies but some immunoreactive fibers were noticed in corpus callosum, commissura anterior, capilla interna and spinal fasciculi. To identify the pro-NGF-LI cell bodies, the IHC procedure was combined with the retrograde transport of a WGA-apoHGF-Gold complex (Bensaab and Mesneye '87) injected in thalamus, hippocampus and spinal cord; results provide evidence that the pro-NGF-LI is localized within neuronal cell bodies. Studies using antibodies against pro-NGF are in progress.


It has been shown that nerve growth factor (NGF) enhances survival of cholinergic neurons in basal forebrain both in vivo and in vitro. Although NGF stimulates different mRNA species, it has been shown that NGF, when added to NGF-R deficient PC12 cells, delayed the appearance of new neurites or labeled with fluorescent latex microspheres was counted under a fluorescence microscope. Some of the cultures were stained for GABA-immunoreactivity using rabbit anti-GABA antiserum. We here support the possibility that NGF enhanced the expression of these enzymes and not the survival of the neurons. We have examined the effects of NGF, alone or in the presence of NGF, on chromaffin cells grown in dissociated cell culture for 6 days or more. Cultures were grown with or without PMA (10 ng/ml), NGF (10 ng/ml) or a 75 NGF Collaborative Research on collagen-coated substrates in Medium 190 190-220 charcoaled-fragile cell sen, then scored for the proportion of cells bearing neurons or labeled by a 24 hour exposure to 1H-Adre. Some were processed for immunocytochemistry or scanning EM. Results: Long-term incubation with PMA produced effects similar to those produced by NGF. PMA increased cell number and proliferation, and elicited the outgrowth of neurons, although they were fewer in number and morphologically different from those elicited by NGF. In PMA - NGF, as compared to NGF alone, neuritic outgrowth was enhanced while cell proliferation was reduced, as was also observed when PMA was added for the last 2 days of a 6-day incubation in NGF (Lillen and Claude, in prep.). Cells from animals of different ages responded similarly to PMA. Thus, PMA, possibly acting through PFC, mimics or modulates long-term effects of NGF.

448.8 INJURED SENSORY NEURONS RESPOND TO DELAYED INFUSION OF NERVE GROWTH FACTOR, V.M.K. Verge, P.M. Richards and R.J. Riopelle. Montreal General Hospital & McGill University, Montreal, Quebec, H3G 1A4 and Queen's University, Kingston, Ontario, K7L 3N6.

Approximately one half of the neurons in adult rat lumbar DRG have high-affinity receptors for NGF. To study changes in these neurons and receptors following peripheral nerve injury and subsequent infusion of NGF, histological preparations were prepared from cryostat sections of L5 DRG incubated with radiolabeled NGF. The right sciatic nerve was transected 30 minutes before infusion in one half the animals, NGF was infused at 250ng/hr to the proximal nerve stump from the 21st to 30th days. By quantitative radioautography, miles and nuclei cultured for the period, dye sites on heavily labeled neurons was seen to fall by more than 80% after sciatic nerve transection, through loss in cell volume (50%) and density (40%); in the 60% NGF neurons with high-affinity receptors, delayed infusion of NGF substantially reversed both receptor loss and atrophy. For neurons without high-affinity receptors, cell volume was reduced only 25% by sciatic nerve transection and was not restored by NGF.

As part of the response to axonal injury and possibly because the cell body is deprived of NGF, fewer high-affinity receptors are displayed by sensory neurons. NGF can regulate its functional high-affinity receptor on adult mammalian neurons.
448.9 ACTIVATION OF CELL PROLIFERATION BY NERVE GROWTH FACTOR IN THE ENDOCRINE OTIC VESICLE AND COCHLEOVESTIBULAR GANGLION IN VITRO. J. Regassa, E. K. Shriver Center, Waltham, MA 02254 and U CSD, La Jolla, CA 92037.

Recent findings have shown that Nerve Growth Factor (NGF) activates cell proliferation in inner ear primordia (otic vesicle, OV; cochleovestibular ganglion, CVG). The effects of NGF on cell proliferation and survival in inner ear primordia (otic vesicle, OV; cochleovestibular ganglion, CVG) were studied. OV and CVG were from 72 hr quail embryos (stage 19-20), containing active proliferating cells for these structures. They were incubated for 24 hr at 37°C in M-199 serumless medium containing 3H-thymidine (4 µCi/10 µg/ml). Duplicate cultures also contained either basic fibroblast growth factor (bFGF) or NGF, or serum plus NGF. Incubation with either NGF or serum resulted in a significant increase in the TCA precipitable incorporation of 3H-thymidine in OV and CVG, as compared to the control in serum-free conditions (5x and 4x for OV; 6x and 6x for CVG). These observations indicate that NGF and serum cause specific increases in 3H-thymidine incorporation in both OV and CVG. For NGF, this effect appears to occur by increasing cell proliferation, and not cell survival, since NGF is still able to elicit its full response in growth-arrested OV and CVG. Supported by grants from the NSF (BNS-8959101), March of Dimes (1#-1090), and Strykeron Foundation.

448.10 CHARACTERIZATION AND LOCALIZATION OF NERVE GROWTH FACTOR RECEPTORS IN THE ENDOCRINE OTIC VESICLE AND COCHLEOVESTIBULAR GANGLION IN VITRO. J. Regassa and J. Regassa, Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

The effects of NGF on cell proliferation and survival in inner ear primordia (otic vesicle, OV; cochleovestibular ganglion, CVG) were studied. OV and CVG were from 72 hr quail embryos (stage 19-20). Identical flasks of active proliferating cells for these structures, were incubated for 24 hr at 37°C in M-199 serumless medium containing (100 µg/ml NGF; 10 µg/ml bFGF) and NGF, or serum plus NGF, or serum alone. Incubation with either NGF or serum resulted in a significant increase in the TCA precipitable incorporation of 3H-thymidine in OV and CVG, as compared to the control in serum-free conditions (5x and 4x for OV; 6x and 6x for CVG). These observations indicate that NGF and serum cause specific increases in 3H-thymidine incorporation in both OV and CVG. Supported by grants from the NSF (BNS-8959101), March of Dimes (1#-1090), and Strykeron Foundation.


Histological and biochemical studies have shown that neurons develop in vivo and in vitro experiments indicate that nerve growth factor (NGF) modulates the levels of choline acetyltransferase activity in cholinergic neurons of the basal forebrain. Previous reports have shown that the activity of the choline acetyltransferase enzyme is increased by the addition of NGF to the culture medium. In these experiments, NGF increased the levels of acetylcholine in the basal forebrain region, in which three morphologically distinct cholinergic cell types: stellate, pyramidal, and bipolar, are found (Kivard et al., 1996). Dissociated cells were prepared from the basal forebrain region of 1-2 day old mouse pups and grown for 7 days following a single addition of NGF, 56 hours after plating. The maximal effective dose of NGF was found to be 200 ng/ml which produced a 1.5-fold increase in the total number of CAT positive cells/well and the number of stellate cells/well was increased by 50%. The addition of 50ng/ml NGF increased the number of pyramidal cells from 12 to 8 x 10^3 cells/well. However, no change in the number of bipolar cells was noted at any concentration of NGF tested. The treatment of PC12 cells with NGF resulted in an increase in the number of cells that were positive for the marker. Infection of these B-gal-PC12 cells or the mouse PC12 line with either NGF or NGF receptor cDNA indicates that the NGF receptor is not expressed in either of these cell types. The fact that NGF and EGF stimulate different patterns of S6 incorporation in these cells indicates that these two peptides induce their full response in growth-arrested OV and CVG. Supported by grants from the NSF (BNS-8959101), March of Dimes (1#-1090), and Strykeron Foundation.

448.12 AUTOCRINE DIFFERENTIATION OF R A T PHEOCHROMOCYTOMA PC12 CELLS USING A RETROVIRAL NGF VECTOR. M.P. Short*, M.B. Rosenberg, D. Erezdine*, F.H. Gage, T. Friedmann* and X.O. Breakfield. E.K. Shriver Cit., Walhain, MA 02254 and UCSD, La Jolla, CA 92037.

PC12 cells have been genetically modified by use of replication-defective retroviral vectors containing either the bacterial gene for ß-galactosidase (β-Gal) or the cDNA for mouse beta-NGF, and the bacterial gene for neomycin resistance. Using the β-gal vector, clonal lines of PC12 cells were obtained in which almost 100% of cells stably expressed this histochemical marker. Infection of these PC12 cells or the original PC12 cells with the NGF vector resulted in extensive neurite formation, which occurred within hours after infection and was maintained for weeks in culture. A majority of cells expressing neurite outgrowth was comparable to that of PC12 cells treated with exogenous NGF. Neurite formation could be blocked by antibody to NGF. The amount of NGF in cells and that released into the medium was assessed by a two site radioimmunoassay and by a bioassay using "naive" PC12 cells. Genetically modified PC12 cells are being injected stereotactically into the CNS of newborn rats to analyze their survival and effects on the brain are being assessed histologically.
448.15 EFFECTS OF NERVE GROWTH FACTOR ON CYTOSKELETAL GEN EXPRESSION IN THE ADULT RAT DORSAL ROOT GANGLION (DRG) NEURONS. M.M. Obringer and J.W. Wong.* Dept. Cell Biology and Anatomy, Chicago Medical School, N. Chicago, IL 60064

While adding NGF does not appear to promote NGF for survival, NGF is thought to be important for the maintenance of normal morphology and physiological function of these cells. In fact, the loss of trophic support from factors such as NGF as a result of axotomy has long been speculated to be involved in the injury response and neuronal degeneration. As a result, a major component of the axotomy response in DRG neurons is the cytoskeletal response. Recent studies by this and other laboratories have suggested that the development of processes, the downregulation of neurofilaments (NF) proteins and mRNA levels and an upregulation of tubulin synthesis and mRNA levels. In the present study, we asked whether the administration of NGF would alter the cytoskeletal gene response in axotomized DRG neurons. To examine this question, the sciatic nerves of adult rats were selectively transected at the middle level and placed in a silicone chamber which was connected to an implanted osmotic pump (Alzet). The transection was performed between 7:00 and 9:00 AM on days 0.5 or 12 days with subcutaneous NGF (0.5 mg/ml) or sterile saline. At 12 days, the axotomized and the contralateral L4 and L5 DRGs were harvested. The L4 DRGs were labeled for 1 hr in vitro with 35S-methionine and the newly synthesized proteins analyzed by quantitative 2D gel electrophoresis/fluorography. The L3 DRGs were embedded in paraffin, sectioned at 10 µm and hybridized with [35S]mRNA probes to the mRNAs of NF (provided by Dr. N. Cowan, NYU) and beta tubulin (provided by Dr. S. Farmer, Boston U). The quantitative results of both protein synthesis and in situ hybridization experiments indicated that NGF treatment did not alter the axotomy response with respect to the major cytoskeletal proteins. DRGs treated with NGF for 12 days after axotomy exhibited a substantial reduction in NF synthesis and NF mRNA levels and a substantial increase in tubulin synthesis and beta tubulin mRNA levels; the level of change in all measured parameters was not significantly different from that observed in saline treated axotomized controls. We conclude that this paradigm of administering NGF to axotomized DRG neurons does not alter the cytoskeletal component of the axotomy response.

448.16 DETECTION OF A PROTEIN KINASE ACTIVATED BY NERVE GROWTH FACTOR IN A HUMAN EWING'S OSTEOSARCOMA CELL LINE. C. Voloneti* and L.A. Greene Department of Pathology, Columbia University P&S, New York, N.Y. 10032

It has been previously found that several human Ewing's osteosarcoma cell lines possess NGF receptors (Thomson et al. 1988 Exp. Cell Res. 174: 333). One of these, the IARC-EW-1 line, responds to NGF by rapid induction of the c-fos proto-oncogene (Thomson et al. 1988). More recent studies reveal that although this line fails to show certain responses to NGF seen in PC12 cell cultures, such as alteration of proliferative behavior upon NGF administration of a series protein kinase (PK) activity (designated PKN; Rowland et al. 1987 J. Biol. Chem. 262:7504). Partial purification of PKN from IARC-EW-1 cells by FPLC on a Mono-S column reveals at least 10-fold activation of PKN by NGF. This activation of NGF by NGF for may be dependent for detection of NGF responsiveness, especially in cells lacking macroscopic responses to the factor.

NEURONAL DEATH II

449.1 AN APPARENT ABSENCE OF CELL DEATH AMONG SPINAL CORD INTERNEURONS IN THE CHICK EMBRYO. S.C. Massey* and R.M. Oppenheim (SPON: R.I. Troost). Dept. of Anatomy, Lake Forest College, Lake Forest, IL 60045

Although naturally occurring neuronal death has been observed in a wide variety of neurons and species, there are reports in the literature indicating that such occurrences of neuronal death are, if any, rare. This is because although several theories have been proposed to account for this phenomenon, the evidence is conflicting. The questions how the spinal cord interneurons die during development, we have examined (i) the section of the lumbal spinal cord at E15.5 and E17.5, and (ii) the spinal cord of E5.5-6.5 embryos in situ. In the latter case, the spinal cord was disconnected from the rest of the embryo and fixed in situ with paraformaldehyde. The embryos were then dehydrated, embedded in paraffin, and sectioned at 10 µm. The sections were stained with hematoxylin and eosin or by the avidin-biotin-peroxidase method. The sections were examined by light microscopy. The results of these experiments indicate that there is no apparent death of interneurons during development. The possible mechanisms by which grafts of catecholamine secreting cells alleviate the signs of experimental parkinsonism are still elusive. We undertook this study to evaluate the possibility that such factors may protect the host cells. In addition to intracerebral transplantation of adrenal medulla, might play a role in these improvements. Aged male Sprague-Dawley rats received 10 µg of alpha-adrenergic drug (0.5 mg/ml) or sterile saline. After several weeks during which they were evaluated for aminophylline-induced rotation, the rats were transplanted with adipoic tissue and received intracerebral infusion of saline or nerve growth factor (NGF). One group received only NGF for a month period. Behavior induced significant reduction in Apo-induced circling in the group treated with NGF + adipose tissue but not in the other groups. 6-OHDA caused significant decrease in the ipsilateral strata. Adipose tissue transplantation exacerbated these changes in 5-HT and 5-HIAA. NGF significantly attenuated the decreases in the 5-HT system without affecting the dopamine system. These results suggest that behavioral improvements seen after adrenal transplantation may be related to other factors in addition to the secretion of dopamine in the denervated nigrostriatal pathway.


Nerve growth factor (NGF) regulates neuronal cell death during development. The PC12 line is a useful model of a nerve cell that provides protection to PC12 from hydrogen peroxide, H2O2, a well known hydroxyl radical generator. Exogenous catalase also protects and NGF does induce nerve growth factor (NGF) like most neuronal lines, have low endogenous catalase levels, NGF protection is abolished by a catalase inhibitor. One possibility is that the NGF and conditioning lesion paradigm that uses a sublethal peroxidative insult to stimulate proliferation, change in morphology, survival in serum-free media. Both NGF and the conditioning lesion itself independently confer cytoprotection from H2O2; together they give a synergistic effect. The use of NGF and conditioned cells display an acceleration of neurite outgrowth compared to unconditioned cells. The PC12 cell line was used to test if NGF could facilitate the quantitation oxidant-antioxidant balance. Supported by NIH grant NS-18708.
449.3

Hypoxic neuronal injury in culture can be ameliorated by pharmacologic blockade at the NMDA gated ion channel. It has not been determined that inhibition of neuronal calcium accumulation accounts for this neuroprotective effect. We have evaluated the effects of calcium and sodium channel modulators on hypoxia-induced neuronal calcium accumulation and injury in culture. Two week old rat cortical neuronal cultures were exposed to hypoxia (5% O2, 95% N2, and 5% CO2) at 37°C for 4 hrs in the presence or absence of ion channel modulators. After the hypoxic exposure neuronal 45Ca++ uptake was measured. Sister cultures were returned to a normoxic environment (95% air, 5% CO2) at 37°C for an additional 20 hrs and were examined for morphological injury after immunocytochemical staining with HRP linked neuron-specific enolase antibody. The percentage of injured cells was calculated by comparing photomicrographs of identical microscopic fields taken before and after hypoxic exposure. Phenytin, tetrodotoxin, verapamil, and diltiazem were found to block Ca++ accumulation yet did not prevent neuronal injury. The inorganic cations, Mg++ and Zn++, which block both voltage dependent and NMDA gated ion channels, inhibited Ca++ influx and protected the cells from morphologically assessed injury.

449.4
SURVIVAL OF CORTICOSPINAL NEURONS AFTER AXOTOMY IS TEMPORALLY CORRELATED WITH GROWTH OF THEIR AXONS INTO SPINAL CORD TARGETS. M. Meriine* and K. Kalil. Dept. of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.


To resolve these discrepancies, we retrogradely labeled corticospinal neurons with bilateral injections of rhodamine beads into different levels of the spinal cord, and 3-48 hours later made unilateral pyramidal tract lesions. Labeled neurons in the contralateral cortex served as controls. Neurons projecting to the lumbar cord completely degenerated if the pyramidal tract was cut before 10 days postnatal. Lesions at 14 days or older resulted in cell survival, although they were somewhat shrunk. In contrast, neurons projecting to the cervical spinal cord survived lesions of the pyramidal tract at 9 days of age, but lesions at 7 and 5 days produced progressively greater but by no means complete cell death. Short survival times used in some of the experiments resulted in incomplete cell changes as early as 24 hrs after axotomy.

These results show that the age at which the lesion occurs determines the ability of corticospinal neurons to survive axotomy. Since corticospinal axons begin to innervate the cervical cord at 6 days and the lumbar cord at 10 days, the ability of corticospinal neurons to survive lesions of the pyramidal tract is temporally correlated with target innervation. (Supported by NIH Grant NS-14428)

449.5

The lateral motor column (LMC) neuron population of the developing frog is under the influence of both limb and thyroid hormone. Although neuron loss is temporarily retarded following limb amputation at the onset of dramatic LMC reductions, by the end of the larval period a substantial reduction in neuron number occurs. Since thyroid hormone influences spinal ventricular cell proliferation, whether limb amputation and thyroid hormone might interact in determining the LMC neuron number outcome in Rana pipiens tadpoles. Treatment with thyroid by immersion resulted in an LMC with somewhat more neurons than controls. When hindlimb amputation is accompanied by continual thyroxine treatment, the LMC of the amputated side exhibits extraordinary numbers of neurons at the time of metamorphic climax. In fact, the neuron counts often exceeded those present at the time of amputation, when the maximum number was normally present. It is suggested that neuron loss was being inhibited as expected, though perhaps for an extended time, while the rate of ongoing proliferation as determined by ventricular mitotic counts was accelerated by thyroxine, as was overall larval development. 3H-thymidine autoradiographic analysis should confirm if the LMC neuron increase, in part, is a result of the thyroxine-enhanced mitotic activity.

449.6
MORPHOMETRIC ANALYSIS OF SCIATIC NERVE MOTOR NEURONS 20 WEEKS AFTER T-9 SPINAL CORD SECTION. E.R. Feringa, R.L. McBride and J.K. Williams*. V. A. Medical Center and Medical College of Georgia, Augusta, GA 30910.

Functional recovery after spinal cord injury is dependent on the condition of neurons deprived of input by the trauma. We previously reported no loss of sciatic motor neurons 10 weeks after T-9 spinal cord transaction and now we extend our observations to 20 weeks after transaction.

We transected the spinal cord at T-7 in anesthetized seven-week old female rats. Ten weeks later, the right sciatic nerve of these rats and 11 controls was severed above the popliteal fossa and the proximal cut end soaked for one hour in a 2% solution of the retrogradely transported fluorescent dye Fluoro-Gold. The rats were perfused 4 days later. Labeled neurons were counted on every fifth 30 µm section and measured on two sections from each rat.

In contrast with some earlier reports, we found that, at least as long as 20 weeks after T-9 transaction, there is no significant change in sciatic motor neurons in number or size. In addition, the transected neurons retain their ability to retrogradely transport Fluoro-Gold. Supported by the V.A. Medical Research Service.

449.7

Although delayed death of corticospinal and rubrospinal neurons after T-9 spinal cord transaction in adult rats was indicated by reduced anterograde (HRP) and retrograde (fluorogold) labeling, the numbers of corticospinal axons at C-1 and T-1 did not decrease. To further investigate the death of neurons proximal to the soma, the spinal cords of 15 anesthetized seven-week-old female rats were completely transected at T-9 and allowed to degenerate for 20 weeks (8 controls) or 20 weeks (8 transected, 9 controls) later, a cotton pellet soaked in a 2% solution of the retrograde fluorescent dye Fluoro-Gold was inserted into a new transaction at T-1. Four days later the rats were perfused. The mean size of labeled red nucleus neurons in transected rats was decreased compared with controls in both 10 and 20 week groups (p<0.01). We conclude that some uptake/transport mechanisms remain intact following axotomy. Supported by the V.A. Medical Research Service.
449.9

In homozygous staggerer (sg/sg) mice, cerebellar Purkinje cells are reduced in number by 75% due to an intrinsic effect of the mutation on Purkinje cell development. In addition, 60% of the normal number of inferior olivary neurons are lost during postnatal development. To determine whether the olivary cell death is cell-autonomous (or caused by extrinsic factors such as the loss of most of the postsynaptic Purkinje cell target) chimeras were constructed from sg/sg Purkinje cell and C57Bl/6 mouse. 5-HT+5-HT (low β-glucuronidase activity) and 5-HT-Gal/Galβ (low β-glucuronidase activity) embryos. Three adult staggerer chimeras were identified. Despite the presence of both genotypes in the olive, cell counts revealed more sg/sg genotype neurons present in the chimeras than in a homozygous mutant. Since sg/sg oligodendrocytes can be “rescued” in the chimeras, their death must be an indirect consequence of sg gene action. Unexpectedly, heterozygote mice (+/sg), while behaviorally normal, lost 30% of their olivary neurons by 6 months of age. We have performed further Purkinje cell counts in 12 month old sg/sg animals and find a 30% loss of these neurons as well. Combined with data from the chimeras, these results suggest that the pattern of staggerer gene action is the same in both the heterozygote and homozygote animals.

Supported by the NIH (NS-20591 and NS-ERB1), the March of Dimes Birth Defects Foundation, and a FYSSEN Foundation Fellowship to HS.

449.11

Neuronal death is a widespread phenomenon that affects many neurons in the developing nervous system. We used an immunohistochemical reaction to identify dying neurons in the subplate of the developing rat neocortex. Rats, 0, 2, 5, and 30 days after birth were perfused with paraformaldehyde. Cortical tissue was reacted with a monoclonal antibody directed against Alz-50; Alz-50 is a 68K protein isolated from the brains of Alzheimer patients (Wolszon et al., Science 230: 649, 1986). Alz-50 immunoreactivity was visualised with a biotin-avidin-peroxidase technique. In neonatal reactive neurons with round cell bodies were in the upper subplate and fusiform Alz-50-positive cells were distributed in the lower subplate. By postnatal day (PD) 5, the Alz-50 immunoreactivity in the subplate had disappeared. The time of origin of subplate neurons was determined using [1H]thyidine autoradiography. These neurons were born on gestational day (GD) 12. Heavily-labeled neurons were still evident on PD 5, but by PD 30 they have disappeared. Using a method which combined Alz-50 immunohistochemistry with [1H]thyidine autoradiography, it was determined that many Alz-50-positive neurons were generated on GD 2. Thus, Alz-50 is expressed transiently and early in the process of neuronal degeneration. Funded by DE 07734, AA 06916, and AA 07368.

449.13

Peripheral nerve injury results in prominent alterations in primary sensory neurones whose peripheral branches were located in the sciatic nerve. The reactions observed included transganglionic changes in other systems. Transganglionic labelling from the injured sciatic nerve transection compared with the contralateral side. The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

The results revealed that the neuronal damage to the gracile funiculus ipsilateral to nerve transection could be prevented by methylenoxanmethanol (MAM). This was achieved by combining MAM with the postnatal day 14 alone and the number of whisker afferents (single injection within 24 hours of birth). The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from the area and volume of layers and cell densities.

The results revealed that the neuronal damage was most prominent in the injured side. The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

449.14

Elevated cAMP prevents neuronal death caused by NGF deprivation. Superior cervical ganglia were dissected from embryonic day-21 rats and dissociated into collagenase-digested tissue culture dishes, ranging from one day to 32 weeks. Degenerative changes were found in the ipsilateral gracile nucleus from three days up to 32 weeks after nerve transection. These changes in terminals, process, and cell body distribution are modified by agents that have previously been shown to alter the death of neurons in other systems. These include the GRACILE PROJECTING PRIMARY SENSORY NEURONES FOLLOWING PERIPHERAL NERVE INJURY IN THE RAT. R.K. Pearson*, H. Aleskogius*, A. Jander**, and A. Holmberg*. Dept. of Anatomy, Karolinska Inst., 104 01 Stockholm, Sweden.

Peripheral nerve injury results in prominent alterations in primary sensory neurones whose peripheral branches were located in the sciatic nerve. The reactions observed included transganglionic changes in other systems. Transganglionic labelling from the injured sciatic nerve transection compared with the contralateral side. The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

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449.12

We have examined the ability of post-hypoxic treatment with MK-801, a potent N-methyl-D-aspartate antagonist, to protect against hypoxic-ischemic brain damage in the neonatal rat. Both carotid arteries of 7-day-old rats were ligated. After 4-6 h, pups were exposed to 8% O2 for 1 h. One group (n=8) received 10 mg/kg MK-801 (i.p.) just after the hypoxic insult. A second (n=8) received saline. Histological examination of the brains 3 days later revealed significant protective effects of MK-801. The percentage of necrotic area was scored as 0, +1 (1-25%), +2 (26-50%), +3 (51-75%) and +4 (76-100%). Post-hypoxic MK-801 reduced the degree of necrosis from +3.50 to +1.12 to +0.05 (p<0.05) in the neocortex, and from +2.10 to +0.03 to +0.05 (p<0.05) in the basal ganglia. Protection was not selective and included necrotic astrocytes. The data suggest that MK-801 has the therapeutic potential to promotes even when given after the hypoxic-ischemic insult.

449.10

Interactions between target and afferent neurons (or peripheral systems) are believed to generate a relatively precise neuronal circuit through tansneuronal cell death. Two models of developmental dysgenesis are reduced by methylazoxymethanol (MAM) in rats were used to reduce the number of Purkinje cells as targets (a single injection on gestational day 14) and the number of whisker afferents (a single injection within 24 hours of birth). The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

The results revealed that the neuronal damage to the gracile funiculus ipsilateral to nerve transection could be prevented by methylenoxanmethanol (MAM). This was achieved by combining MAM with the postnatal day 14 alone and the number of whisker afferents (single injection within 24 hours of birth). The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

The results revealed that the neuronal damage was most prominent in the injured side. The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.
449.15
NEURONAL DEATH CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION FROM CYTOSINE ARABINOSIDE (ARA-C) RESEMBLES DEATH CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION. T.L. Wallace, D.P. Martin, and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110

We have characterized in vitro a model of trophic factor deprivation in which we have previously shown that neuronal death requires RNA and protein synthesis. Comparison of the dose response curves of cycloheximide for inhibition of protein synthesis and requirements for RNA and protein synthesis. Comparison of the dose response curves of cycloheximide for inhibition of protein synthesis and protein synthesis ending in the production of new proteins which kill the cell.

449.16
NEURONAL DEATH CAUSED BY CYTOSINE ARABINOSIDE (ARA-C) RESEMBLES DEATH CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION. T.L. Wallace, D.P. Martin, and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110

We report here that Ara-C causes the death of rat sympathetic neurons in a fashion resembling that caused by NGF deprivation. Superior cervical ganglia were dissected from embryonic day-21 rats and dissociated onto collagen-coated tissue culture plates. After a week in the presence of NGF they were exposed to Ara-C (10^-5M). No changes were observable until about three days, whereupon the neurites began to thin and fragment. Over the next 24 hours the cell bodies became condensed and phase dark, such that by five days after addition of Ara-C all neurons in the dish were completely destroyed. The morphological and temporal characteristics of cell death that began around three days after adding Ara-C were very similar to those observed starting 24 hours after NGF deprivation. Although Ara-C is an antimotic drug, its action here does not involve DNA synthesis because these neurons are postmitotic and unharmed by any other antimitos (Ara-A, Ara-T, and FUdR). As with neuronal death caused by NGF deprivation, the death caused by Ara-C was prevented entirely by inhibitors of protein and RNA synthesis, agents which elevate intracellular cAMP, and elevated concentrations of potassium in the culture medium. These data suggest that Ara-C causes an active death of neurons similar to that caused by trophic factor deprivation.

450.1

Attention to the topography of shark retina has been hindered by the obsolete view that the elasmobranchs as a rule possess all-rod retinas. After Gruber et al. (Vision Res. 3:397, 1963) demonstrated conclusive evidence of cones in the lemon shark, at least 24 species from 10 families of elasmobranchs with duplex retinas have been described. In this study, unstained retinal wholemounts and Nomarski DIC micrographs were used to obtain the topographic organization of cones and ganglion cells in juvenile lemon shark retina. Retinal cell distribution is organized into an irregular system in register with the retinotectal streak, which is oriented within about 15° above and 15° below the visual axis. Examination of Nissl-stained cells within the ganglion cell layer reveal a peripheral density of 130 X 10^4 cells/mm^2 counted in wholemount material compared to 15.0 X 10^4 cells/mm^2 counted in tangential sections. Peripheral densities drop to 4.5 X 10^4 cells/mm^2. To assess the proportionality of this with the different isodensity contours we counted an estimated 1,220,000 - 1,570,000 (mean 1,430,000) ganglion cells/mm^2 within a specialized region of unmystified axons in the retinal apex. The unmystified axon population (20%) follows closely the topography of the total population of axons. A total of 104,652 axons were found in the optic nerve compared to 102,918 cells situated in five sublaminae within the retinal ganglion cell layer. A direct relationship is revealed between ganglion cell soma size and axon area. The corresponding topographic organization of the retina and optic nerve may reflect some functional mimicopy.
450.5


Anatomical and physiological studies of the cat retina provide estimates of the limits of spatial resolution at various retinal eccentricities. Estimates based on the density of ganglion cells, dendritic field diameters and receptive field diameters suggest that X (magnocellular) and Y (parvocellular) functions are spatially segregated in the cat. Such predictions for areas outside of the central retina have never before been verified by psychophysical testing. We developed procedures that enabled us to behaviorally measure visual function at any retinal eccentricity. We measured grating acuity in cats, whose eye position was stabilized by a Kopf system of a small camera coil technique. During behavioral testing, the cat was placed in an apparatus equipped with a head restraining device and two adjacent response pedals. During each trial the cat was required to maintain fixation on a laser spot and respond to the presence or the absence of a grating by pressing the right or the left pedal. The cat readily adjusted to these training conditions and we were able to measure acuity along the horizontal and vertical meridians at eccentricities up to 12 deg in the nasal, temporal, superior and inferior retina. Acuity in area centralis reached about 4 cd/deg and it declined by a factor of two at 4 deg and a factor of 3.4 at 12 deg eccentricity. The acuity was higher in the nasal than temporal retina. At all eccentricities the behavioral acuity exceeded the resolution limit derived from V(alpha)-cell properties, but was consistent with the values derived from the X (beta) cell properties. Supported by T22 NSH5260, BY00175, BY00319.

450.7


We have previously found, in acrylamide-exposed macaques, selective degeneration of those retinal ganglion cells that project to parvocellular layers of the lateral geniculate nucleus (LGN). In the squirrel monkey, it is possible that spatial mapping of the visual system of a New World monkey, the squirrel monkey and macaque share some feature which makes them vulnerable to this chemical. The selective lesion of the parvocellular pathway reported here is similar to lesions in the cat and monkey that were produced by the injection of herpes simplex virus into the lateral geniculate nucleus of the macaque. We are currently testing this hypothesis at the electron microscopic level. Supported by NIH grants NS 51865, ES 01247, and ET 01319.

450.9


Studies were initiated to assess the organization of parallel pathways from identified retinal ganglion cells (Gs) to the superficial layers of the superior colliculus (SC) in rabbit. In cat, Gs-Gs provide crossed projections (Fukuda and Stone, '74) to the upper stratum griseum superficiale (SSS) (Berson, '87), while Y-Gs provide both crossed and uncrossed projections (Fukuda and Stone, '74) to the deeper portion of the SSS (Hoffman, '73; Mcllwain and Lufkin, '87). It is possible that different functional types of Gs have different sublaminae and hence parallel projections to the superior colliculus. In the squirrel monkey, it is possible that functional types of Gs have different sublaminae and hence parallel projections to the superior colliculus. We are currently testing this hypothesis at the electron microscopic level supported by NIH grants EY-01730, EY-02923 and the E.K. Bishop Foundation.

450.6


The variation of macaque visual acuity with eccentricity was mapped behaviorally from the fovea to 30 deg eccentricity along nasal and temporal horizontal meridians. These results can be interpreted in terms of retinal eccentricity which change with eccentricity such as optical quality, cone density, ganglion cell density, and dendritic and receptive field dimensions. Acuity was measured by the discrimination of verticals from horizontal sinusoidal grating (17 cd/m2 mean luminance) to minimize the use of alligned information. Fixation locus was controlled behaviorally and monitored with scleral search coils. Acuity was measured at 0.3, 6, 12, 20, and 30 deg eccentricity in an interleaved series, and stimulus size was approximately 10 cycles of a just resolved grating. Visual acuity decreased more sharply across temporal than nasal retina, and this difference was slightly greater than that seen in humans. The rate of decline was consistent with cone density over the central 10 deg of the retina and with the density of P ganglion cells at greater eccentricities, although it has been shown that such densities cannot in themselves limit acuity. Comparison of our acuity results with dendritic and receptive field dimensions suggest that spatial averaging may play some role in limiting visual acuity. Supported by grants NSF BNS-8518585, ES 01247, and EY 01319.
AFFERENT AND EFFERENT CONNECTIONS OF THE ISTMIC-ONOCULAR NUCLEUS IN PIGS (Columba livia).

Woodson, W. T., Shimizu, H. and H. Karten. Department of Neurosciences, M-008, School of Medicine, University of California, San Diego, La Jolla, CA 92037

Previous studies (McGill et al., '66, 'b) have shown that the isthmo-oculomotor nucleus (ION) is the source of a projection (centrifugal) to the retina, and that a topographic projection from the optic tectum. The afferent and efferent connections of this nucleus were identified using Phaseolus vulgaris leucoagglutinin (PHA-L). Retrogradely labelled cells were found at the margin of layers 9-10 throughout the rostro-caudal extent of the optic tectum. The dendrites of these tectal neurons extended from the rostro-caudal aspect of layer 2-7. Efferents from ION terminate in the contralateral IPL. Centrifugals to the retina arborize in layer 5b and, more sparsely, in layer 5b of the inner plexiform layer (IPL). Many processes could be followed into the inner nuclear layer, forming pericellular nests and "palmate" endings around somata of medium-large sized amacrine cells. These results indicate that, in addition to direct supernumerary upon somata of amacrine and displaced ganglion cells, the centrifugals also terminate within sharply defined laminae of the IPL. Furthermore, the organization of the isthmo-oculomotor efficients suggests that the optic tectum is able to exert bilateral control over discrete subpopulations of amacrine cells in the retina. Support by NIH-EEY06890 to HJK and the Ford Foundation to WW.

AXON COLLAGERALS AND/OR EFFERENT FIBERS IN THE MONKEY RETINA. C. Ueta*, S. Bisti,*and S. Vallerga*. (SPON: European Brain & Behavior Society.) 1st.CIB. Biofisica,CNR, 16146 Genova and 1st. Neurofisiol.CNR, 56100 Pisa, T/A

The presence in the monkey retina of long axons with many collaterals ending as knobbed branches at the surface of the inner nuclear layer (INL), has been reported by Perry et al. (Neurosci. 12:1101,1984) after injection of HRP in the optic nerve. Those collaterals were tentatively assorbed to centrifugal fibers.

In reduced silver stained retinas of Macaca fascicularis we see fiber branching often, emerging from axon bundles, resemble the fibers reported in HRP material. In one instance we could trace the origin of collaterals to the axon of a small neuron with large dendritic field, located in the ganglion cell layer of the monkey retina. The collaterals travel for several mm. through the inner plexiform layer (IPL), keep preferably orthogonal to axon bundles, reach on occasion the INL, and cover mostly the nasal region. Therefore both centrifugal fibers and ganglion cells arising from the inner nuclear layer of macaque may be present in monkey retina.


We have used somatostatin-like immunoreactivity (SRIF-I) in the adult cat retina using a mouse monoclonal antibody (from Dr. A. Buchan) directed to SRIF-I. Of the retinal cells, representative of 8 others, was carried out. Two distinct groups of SRIF-I somata, both distributionally different in retina, were found. The first group consisted of large cells with granular staining neurons and poorly stained primary processes. There were 477 such cells, i.e. in the ganglion cell layer (GCL). Their density ranged from 0.2 cell/mm² in superior retina to an average of 4 cell/mm² in inferior retina. Density peaked at 11 cell/mm² in a region about 4 mm inferior to the area centralis. The average soma area of a sample from inferior nasal retina was 940 ± 200 µm². An analysis of cell distribution in this region found the pattern to be non-random (chi square test, p<0.01) with an average nearest neighbor distance of 347 µm.

The second cell type was characterized by dark stained, small to medium somata with 2-4 primary processes. There were 1,782 such cells in the outer retina, except at the retinal margin, where density was high. The average soma size in inferior nasal retina was 297 ± 22 µm² for cells in the GCL and 173 ± 22 µm² for cells in the INL. An analysis of the distribution of cells in this region found the GCL cells to be distributed non-randomly (chi square test, p<0.01) with an average nearest neighbor distance of 228 µm. (Supported by NIHCDS T32 NS07300, EY00991 and EY00467.)

MORPHOLOGICAL STUDIES OF DISPLACED GANGLION CELLS IN THE CHICKEN RETINA. G. Yang*, T.J. Millar* and I.G. Morgan, Center for Visual Sciences, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. 2601, Australia.

About 4000 displaced ganglion cells (DGCs) were detected in the chicken retina by back-labeling with fast blue injected into the neuroretinal core. Correlated with a section in the optic nerve. Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20 to 30um peripherally. After injections of Fast blue in intraretinal injections in the optic nerve into intraretinal injections in the optic nerve. Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20 to 30um peripherally. After injections of Fast blue in intraretinal injections in the optic nerve. Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20 to 30um peripherally. After injections of Fast blue in intraretinal injections in the optic nerve. Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20 to 30um peripherally. After injections of Fast blue in intraretinal injections in the optic nerve. Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20 to 30um peripherally.

We have previously reported on development and analysis of a simple mathematical model for formation of ocular dominance columns in mammalian visual cortex (S. New. 1984. J. Neurosci. Abstr. 12:1878) (1884). The model provides a common framework in which a variety of activity-dependent phenomena, such as ocular dominance columns and activity-dependent release and uptake of trophic factors, can be studied.

We report here results of an LGN linearspace morphometry analysis of ocular dominance columns. The LGN linearspace morphometry analysis is generated by selecting 25 X 25 grids, constructed via a 7 X 7 array to the width of the arbor space within an area of 6,210 X 2,360 (a reasonably complete field). If either one of the columns, as determined by the computer program, is intersected by a grid point, the resulting column is then accurately predicted by our previous theoretical results. The results clearly demonstrate that the spread of the optical signal might be due to electrotonic coupling of glial cells, which we can define as the mapping that best meets the above constraints, and in this case the mapping that best meets the above constraints, and in this case is then optimal in the following way: First, we can define a new approach to optical imaging that can be used to detect activity of neuronal assemblies, and that can be used to detect activity of neuronal assemblies by blocking synaptic transmission. Stimulation in WM evoked signals which were restricted to the upper three layers. Thus, it is possible to detect activity of neuronal assemblies, and that can be used to detect activity of neuronal assemblies. We started by investigating on-going (spontaneous) activity (without a stimulus). The cat visual cortex was stained with the dye H2-335. The on-going activity was detected continuously for 70 seconds using an incident-light optical system (124 mm), located in a single unit (2 units) recordings. To search for spatio-temporal patterns of coherent activity from neuronal assemblies, we used optical evoked imaging. Using optical evoked imaging, we found that the optical signal reflects the basic functional unit in this cortical area. On-going (spontaneous) activity was detected continuously for 70 seconds using an incident-light optical system. Analysis of the data revealed that the averaged optical signal was restricted to the upper three layers. Therefore, the spatial patterns must be dynamic over a short time scale. Finally, the spatio-temporal patterns appeared more heterogeneous with lighter anesthesia.

Since the amplitude of the on-going activity was small in the previous experiments, and the pattern was dynamic, it is likely that a new approach to optical imaging that can be used to detect activity of neuronal assemblies was used. This is not assumed, observed patterns invariably turn out to be retinotopic in both species. In the monkey, regions representing right and left eye inputs tend to be arranged in parallel stripes, while in the cat, like other species, this is not observed. Therefore, it may play a significant role in cortical function of alert animals.
It has been shown that rat extrastriate cortex can be subdivided into at least 9 separate areas. The connections among these areas were traced using localized injections of WGA-HRP. Accurate placement of injections was guided by the pattern of collateral connections which were revealed in vivo (Malach; Soc. Neurosci. Abs. 11/4). Results indicate that each area is connected to at least five other fields situated both in areas 18a and 18b. In all areas studied, the interconnections appear to relate similar representations of visual space. There seems to be a global map, within which the various fields are embedded, such that extreme rostral areas are connected more to lower field representations in striate and extra-striate cortex, while extreme posterior areas appear to over emphasize the upper visual field. Middle visual areas seem to be connected to all areas roughly equally. Thus, one factor distinguishing the various visual maps in the rat might be a systematic shift in emphasis of different locations within the visual field. Supported by BSR 00258 and Israel inst. for Psychobiol.

The study investigated the distribution and morphology of neurons surviving for up to 8 years in the dorsal lateral geniculate nucleus (LGN) after total unilateral removal of the striate cortex in a strip of 11 rhesus monkeys. All surviving neurons were counted and those from a sample of 5 sections through the central portion, and distribution through the nucleus was plotted. Neurons survived in both laminar and interlaminar zones. Cells surviving within the parvocellular layers are significantly smaller than those in the parvocellular interlaminar zones and those within the magnocellular interlaminar zones. However, there seem to be no differences in cell body size between the magnocellular and parvocellular layers and the two interlaminar zones. In one animal (surviving for 96 months after the striate lesion) horseradish Peroxidase injected into the premitrat cortex retrogradely labelled neurons throughout the degenerated caudal LGNd in topographic register with the central retina and with the position of the premitrat injection sites.

The distribution of cytochrome oxidase (CO)-rich regions was studied in flat-mounts of primary visual cortex (VI) of 2 normal and 2 enucleated Cebus monkeys. Monocular enucleation was performed under pentobarbital anesthesia 4 to 7 months before sacrifice. In the enucleated monkey, the topographic distribution of ocular dominance (OD) stripes in layer IV was similar to that described for macaques (Le Vay et al., Exper. Brain Res. 4(4):486). Stripes tend to intersect the VI/V3 border perpendicularly, and to run along inoscentricty lines within the calcinar cortex. In opercular VI, stripes are more in parallel with the VI/V3 border, except at the foveal representation, where no consistent pattern is observed. In the normal monkey, the most striking aspect of CO blob density. For eccentricities ranging from foveal to 60 degrees, a constant blob density (about 4 blobs/mm²) was observed. In monocular representation, however, the blob density fell to about 2.7 blobs/mm². Mean blob area decreased towards the periphery of VI. In the enucleated monkeys, CO blobs tended to be smaller in regions overlying enucleated-eye domains, but were still visible and clearly outlined, even 7 months after enucleation. Supported by NIH Grants EY06342 and EY 07193.

It has been observed that GABA-immunoreactive (GABA+) neurons in the striate cortex (SC) are not a structurally homogeneous group. In this study we describe the soma area and laminar distribution of GABA+ neurons and of a sub-population of these neurons that are contacted by substance P-immunoreactive terminals at the light microscopic level (GABA+SP+). Sections of the SC were incubated in polyclonal antisera to GABA and SP respectively with the appropriate antibodies, and visualized using different chromogens. Soma area and cell density were determined using a computer-video-microscope system. Cell density of GABA+ cells was highest in layers 2 and 4CB. GABA+SP+ cells were most common in layers 3, 4B, and 6, but were few in 4CB. Soma areas (µm²) of GABA+ cells (n=400) are shown below:

<table>
<thead>
<tr>
<th>Lamina</th>
<th>1</th>
<th>2A</th>
<th>3B-4A</th>
<th>4B</th>
<th>4C</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>58</td>
<td>85</td>
<td>110</td>
<td>118</td>
<td>100</td>
<td>96</td>
<td>110</td>
</tr>
</tbody>
</table>

GABA+ cells in layers 1 and 2 were smaller than those in deeper layers (P<0.05, Scheffe's test). Soma area variability was smaller in layers 1 and 4CB, and large in 4B, 5, and 6. GABA+SP+ cells were on average 1.5 times larger than GABA+SP- cells (P<0.05). In conclusion, this study shows that the size of GABA+ cells in the SC varies in relation to cortical depth, and that SP+ terminals contact medium to large GABA+ cells. It is likely that GABA+SP+ cells overlap with large GABA+ cells shown to label with the VVA lectin. This study was supported by NIH grants EY 01208, EY 04353, and EY 07031.


We have studied the topography of the afferent connections to area 17 with double retrograde label tracing techniques. A graphic method was developed to calculate the extent of the convergence and divergence of afferent connections. The divergence is the extent of an afferent structure which contains neurons converging on a small region of area 17. The divergence is the extent of area 17 innervated by a small region of the afferent structure. The results show that the divergence of the projection from the LGNd to area 17 is 0.4 mm and its divergence 2 mm. The cortical afferents present much larger values of divergence. For example, the divergences of the reciprocal connections between areas 17 and 18 are 6 mm. The divergence of the projection from area 17 to area 19 is 10 mm and those from areas 2a and PMd are close to 20 mm. Knowing divergence and convergence and the retinotopic organizations of areas 17 and the LGNd, we show that the geniculate-audience projection links regions representing the same part of the visual field. Such is not the case, however, for the cortical afferents of area 17. For example, the convergence region in area 18 represents a zone 15° wide in visual field and it converges on a projection line of area 17 which represents 5° of visual angle. These results suggest that, contrary to the geniculate afferents, the connections from the cortical areas preserve parameters other than retinotopy.

INTACT CORTICAL AND CALLOSAL CONNECTIVITY IN THE VISUAL SYSTEM OF THE CAT. J.P. Guilleme1, L. Ritcher*, M. Petto, F. Lepore*, Univ. du Quebec and Univ. de Montreal, Quebec. Anatomical studies have shown that corpus callosum (CC) neurons connect homotopically and heterotopically the visual areas such that the higher order areas project to calcarine or higher order areas. However, the latter are also extensively interconnected intra-cortically. The present study examined with electrophysiological methods, combined with localized inactivation, the organization of these CC connections. Neuronal activity was recorded from CC recipient neurons in areas 17-18 or lateral suprasylvian (LS) extrastriate cortex, from CC fibres in normal cats; from CC fibres in split-chiasm cats. Sequential inactivation of areas 17-18 or ipsilateral LS is possible with topical application of injected aminobenzidine. Relatively short axons radiate from the lateral geniculate complex receive a point-to-point representation of the retina, but project to cortex in a point-to-line fashion. A dorsal-ventral line of geniculate cells represent axons along a vertical meridian, so all cells along the corresponding medial-lateral line of the cortex receive input from all points along that vertical meridian. The results also deals with the way in which cortical cells receive input from all points along the horizontal meridians. Axons in visual cortex of penetrations were labeled in an in vitro whole-brain preparation with focal HRP injections. Preparations were maintained up to 10 hours in Ringer's solution, flattened and reacted with diaminobenzidine. Relatively short axons radiate from the injection sites. The directions of 306 axons relative to the rostral-caudal axis were measured in 9 injection sites. Axons project in all directions, but with some preference along the representations of the vertical meridians. Thus, one of the visual cortical projects in all directions to other nearby parts of the visual cortex; a cortical cell apparently receives input from all points along the horizontal by a series of short, intracortical projections.


Excitatory amino acids (EAs) such as glutamate and aspartate are suggested to be transmitters at least at geniculo-cortical synapses in the cat visual cortex (Tsutomo et al. J. Neurophysiol. 55, 469, 1986). EAA receptors can be classified into three types according to their NMDA-like actions: N-methyl-D-aspartate (NMDA), quisqualate, and kainate receptors. In the in vivo study using slice preparations of the cat visual cortex, we addressed a question of which types of receptors mediates excitatory post-synaptic currents (EPSCs) and EPSPs recorded from layer 1/III cells evoked by electrical stimulation of the underlying white matter. We used Z-aminoo-5-phosphononoate (APV) a selective antagonist for NMDA receptors (KYN) as a broad-spectrum antagonist. An application of KYN through the perfusion medium with 0.2-1.0 mM suppressed EPSCs so that their rising phase and peak amplitudes were dramatic reduced. By contrast, APV (50µM) suppressed the EPSP slopes in some of the cells but it did not affect their amplitudes. There was a tendency that the APV-sensitive EPSPs appeared when bicuculline, a GABAergic antagonist, was added to the medium. This study utilized a tool to study cortico-cortical callosal connectivity.
451.19

ELECTROPHYSIOLOGICAL INVESTIGATION OF CALLOSAL CONNECTIONS OF RAT VISUAL CORTEX IN A SLICE PREPARATION. T. J. Teyle and R. L. Berry (Spon: D. Molfese). Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

In vitro slice techniques have proven powerful tools for the investigation of the electrophysiology of brain circuitry in many brain areas, especially those possessing a lamellar organization. In the present study the slice preparation was used to study the circuitry of the connections between right and left hemisphere visual cortical areas which are mediated by fiber tracts passing through the corpus callosum in a curved trajectory.

Visual cortical slices were obtained from Long Evans hooded rats (2-3 wk) in a standard fashion except that curved cutting blades conforming to the callosal fiber trajectories were employed to preserve these tracts. Depth profiles of field potentials in response to white matter stimulation were recorded and subjected to current source density analysis. The curved slices greatly increased the medial extent of white matter that, when stimulated, produced current sources and sinks in cortical areas OCMN, OCMG, OC1M and the OC1B/OC2L border--in some cases this extended into the contralateral white matter. This finding indicated that the callosal pathways mediated these effects. The patterns of current sources and sinks evoked in these cortical areas by callosal stimulation will be presented. Supported by ONR Grant N00014-86-0644.

451.21


Pyramidal cells in layer 5 of the primary visual cortex (area 17) project to several subcortical targets, such as the superior colliculus (SC), the lateral posterior nucleus (LPN) and the pons. Previous studies have shown that these cells can have axon collaterals to more than one subcortical site. In area 17 of the rat there is an additional projection of layer 5 neurons to the contralateral cortex. We were interested whether different cells in layer 5 project to cortical and subcortical targets. Two fluorescent tracers, fluoro-gold and rhodamine labeled latex microspheres, were injected into the SC and the contralateral cortex respectively. Retrogradely labeled cells from the SC were restricted to layer 5. Callosal projecting neurons were found in all cortical layers near the 17/18 border and in lower layers throughout area 17. No double-labeled cells were detected, indicating that the two projections arise from different cells. There was a wide variation in soma sizes of the labeled cells. Corticothalamic cells had somewhat smaller cell bodies than callosal cells, but there was a considerable overlap between the two populations of neurons. In order to get a more detailed view of the cells’ structure, we combined retrograde labeling with intracellular staining. After injections of microspheres into the SC or the contralateral hemisphere, brain slices were prepared from area 17 and retrogradely labeled cells were injected with Lucifer Yellow. Callosal cells have 4–6 basal dendrites and an apical dendrite terminating in or below layer 1.

Cortical cells possess 6–9 basal dendrites and a prominent apical dendrite which always forms a large tuft in layer 1. Thus, each population of neurons has a rather stereotyped dendritic branching pattern, despite the large variation in soma size. The marked morphological differences between cortical and subcortical projecting neurons in layer 5 suggests that there is an association between structure and function of these cells.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS VIII

452.1


GAP-43 is a phosphoprotein synthetized in high concentrations during development of the mammalian CNS. Despite its prominent role during embryogenesis, relatively little is known of its role in the adult CNS. We have studied the distribution and cellular localization of this protein within brains of developing Sprague-Dawley rats. Numerous pre- and postnatal time points were examined. GAP-43 was first detected in the periphery of whole mount preparations as early as E-10. Subsequently, dense fascicles of GAP-43 positive fibers were detected throughout all prenatal time points in regions corresponding to major fiber tracts. By early postnatal days, the intensity of these labeled fibers decreased. Thereafter GAP-43 immunoreactivity assumed its adult-like characteristics and could be detected within the neuropil of selected anatomical regions.

452.2

EXPRESSION OF GAP-43 DURING CNS DEVELOPMENT: AN IN SITU HYBRIDIZATION STUDY. B. Jacobson and G. Zetterberg, Dept. of Neurology and Genetics, Children’s Hosp., Boston MA 02115

GAP-43 (aka B50, F1, pp46), a developmentally regulated neuronal phosphoprotein found in growth cones and synapses, is believed to play a role in axon growth and/or synaptic plasticity. If GAP-43 is important for process elongation in general, it should be expressed in most, if not all neurons during developmental axon growth. Alternatively, it might serve a specialized function in a subset of neurons. We are studying the distribution of GAP-43 message during development using in situ hybridization. Frozen sections of rat embryos at various stages are hybridized with an 35S labeled RNA probe complementary to the GAP-43 message. Specific hybridization signal is detected autoradiographically. The 1.4 kb GAP-43 message is detectable on Northern blots by 16 days gestation (E16). In situ, no signal is seen in the neural plate (E10) or in neural tube prior to the formation of the intermediate layer (E12). By E13, signal is seen in the primordial motor columns of the spinal cord, the sensory ganglia, retina, and intermediate layer of the brainstem, with especially strong signal in some clusters adjacent to the ventricular layer--presumably brainstem motor nuclei. Later (E15, E16) much of the intermediate layer is labelled, with motor columns and patches of brainstem tegument showing stronger signal than surrounding areas. Thus, GAP-43 expression is widespread in early CNS development, but the level of its expression appears to be heterogeneously distributed.
452.5 GAP-43 PROMOTES AXONAL OUTGROWTH AND REGENERATION. R. J. O'Brian*, R. L. Neve, L. Villa-Komaroff and B. A. Yankner*. Dept. of Medicine, Massachusetts General Hospital, Dept. of Genetics, Children's Hospital, Boston, Massachusetts 02115.

The neuronal growth associated protein (GAP-43) is normally present at low levels in the rat pheochromocytoma cell line PC12 but is induced by treatment with nerve growth factor (NGF). In order to determine if GAP-43 is causally involved in neurite outgrowth, PC12 cells were transfected with GAP-43 cDNA in a DNA expression vector by the calcium phosphate method. Several clones were isolated that overexpressed GAP-43 RNA from the transfected cDNA and exhibited a marked acceleration of neurite outgrowth in response to NGF. The clones with higher GAP-43 RNA expression showed a greater neurite outgrowth response than untransformed PC12 cells. Following mechanical shearing of neurites from differentiated PC12 cells, those cells overexpressing GAP-43 RNA showed a more rapid regeneration of neurites than controls; in addition, these cells displayed transient neurite regeneration in the absence of NGF. These results suggest the active involvement of GAP-43 in axonal outgrowth and regeneration.


The neuronal protein B50 has a molecular weight of 24 kDa determined by direct chemical and cDNA sequencing, but has an apparent molecular weight of about 50 kDa on SDS gels. The protein contains 14 Ser and 14 Thr residues which are potential sites for casein kinase II phosphorylation. Phosphoamino acid determination revealed the presence of [32P]-Ser, but not [32P]-Thr, in an acid hydrolysate of [32P]B50. Furthermore, the hydrolysate of [32P]B50 was also digested by SAP and the reaction mixture was separated by HPLC, with UV and on-line beta radiation detection. The UV chromogram contained two labelled fragments which ran as two 28 kDa and 14 kDa bands on SDS gels. Both are N terminal fragments of B50. Therefore, we conclude that B50 contains a single phosphorylation site at Ser41, since all other Ser residues are eliminated.

The NGF-induced neurite outgrowth in PC12 cells that encodes a novel neuronal intermediate filament protein that is present in PNS and CNS neurons (Leonard et al. J. Cell Biol. 105(1):1988). The present results concern the identification and characterization of this protein. Partial microsequencing of the major cytoskeletal protein of PC12 cells (55 kDa; pI=5.6-5.8), derived by extraction with 1% Triton X-100, yielded a 14 amino acid residue sequence that is identical to a portion of the sequence deduced from the NGF-induced message specifically recognizes the 58 kDa tone-associated growth cone GAP-43. Supported by NIH Grant NS 26091.

452.12 TUBULIN TYROSINE AND SERINE PROTEIN KINASES IN RAT BRAIN GROWTH CONES. M. Cheng1, N. Sahyoun1, and N. Rayboun1. S. Burgess. The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

We had previously described the multi-site phosphorylation of growth cone tubulin on ser and tyr residues. We now present data pertaining to the protein kinases catalyzing these reactions. The Triton X-100-soluble fraction of growth cone membranes contained a ser protein kinase which preferentially phosphorylated the B-subunit of tubulin. The enzyme was retarded on several gel-permeation columns, displayed polydispersive behavior, and thus could be separated from the majority of growth cone polyproteases. This partially purified tubulin kinase associated with an apparent Km of 0.4 µM, and the corresponding values for ATP and MgCl2 were about 23 µM and 2.5 mM, respectively. The enzyme was not inhibited by leupeptin, tubulin protein kinase in the cytoskeletal fraction of growth cones was further confirmed by the selective binding of anti-phosphotyrosine antibodies to phosphorylated cytoskeletal tubulin following electrophoretic transblotting. The cytoskeletal fraction also phosphorylated pol gly-tyr efficiently with an apparent Km of 49,000. The relationship of the ATP-binding protein to the protein kinase is under investigation.


Upon activation by 12-0-tetradecanoylphosphol-13-acetate (TPA) or diacylglycerol, PFC is functionally converted by CANP to a catalytic fragment which phosphorylates other cytoskeletal proteins to generate physiological responses. We report that activation of PFC promotes neurite outgrowth from ganglia explants. To further substantiate the involvement of PFC in this system, the effect of inhibitors of CANP, leupeptin, and a potent inhibitor of PFC, staurosporine on neurite outgrowth were examined. Pretreatment with 20-500 µM leupeptin, but not TPA, reduced the stimulation of neurite fascicles induced by TPA. Leupeptin itself did not have any effect on neuronal development. Staurosporine inhibited neurite outgrowth with an IC50 of 3 nM. At concentrations of 10-200 nM, it was found to exhibit neurite-promoting activities which were additive to those of TPA and were not inhibited by pretreatment with leupeptin. These results suggest that the activation of PFC by CANP is important in the induction of neurite outgrowth. While staurosporine is a potent inhibitor of PFC in vitro, its effect in vivo appears to be complicated by its non-specific nature towards other protein kinases. (Supported by NS21626, NIMH.)
452.15

**INTERACTION BETWEEN ENZYMATIC AND NON-ENZYMATIC MECHANISMS IN CHICK EMBRYO BRAIN NEURONS.** M. Lomme,*, C. Ferguson,*, D. B. Shuparts,*, J. Rosack,*, P. Caracciolo,*, T. Gisi,*, P. Moroder,*, S. R. Levinson**, and M. Ellisman**.

Supported by MRC Canada and the Canadian Paraplegic Association.

**The aggregates were often associated with distinct irregularly shaped areas of neurite growth in these neurons.**

**Intracellular calcium concentration and therefore may influence the rate of neurite growth.**

452.16

**THE PROTEASE TRANSPORT IS INDUCED PRIOR TO NEURITE EXTENSION IN N G F -TREATED PC12 CELLS.** C. M. Machida and C. G. Cimino.

Supported by Cell Biology & Anatomy and the Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

We report here that transin mRNA levels increased dramatically in rat pheochromocytoma (PC12) cells upon stimulation with nerve growth factor (NGF), reaching a maximum level within 24 hours. Although neurofilament (NF-M) mRNA levels also rose with the same time course in NGF-treated PC12 cells, the basal (uninduced) levels of mRNA for this cytoskeletal component were also high. When we compared these biochemical data with the morphological data, we found that transin mRNA levels rose prior to the initial appearance of neurites. Epidermal growth factor (EGF) and basic-heparin binding growth factor (b-HBGF) were unable to elicit both an induction in transin mRNA levels as well as a change to the neuronal phenotype. We also report that transin mRNA induction is not a primary effect of NGF, since cycloheximide was able to block the NGF induction. These data suggest that transin induction is part of the constellation of events induced upon NGF treatment, and may be involved in the process of neurite extension.

452.17

**SODIUM CHANNELS ACCUMULATE ON AXOLEMMA OF HYPEREXCITABLE NEURONS.** P. Caracciolo,*, T. Gisi,*, C. Ferguson,*, D. B. Shuparts,*, J. Rosack,*, S. R. Levinson*, and M. Ellisman**.

Supported by NIH grant NS15070.

**L TYPE CALCIUM CHANNELS MAY REGULATE NEURITE GROWTH IN CHICK EMBRYO BRAIN NEURONS.** M. Lomme,*, C. Ferguson,*, D. Shuparts,*, J. Rosack,*, P. Caracciolo,*, T. Gisi,*, P. Nichols,*, T. A. Auger, and T. A. Auger,*, and G. Audesirk,*, Biology Dept., University of Colorado at Denver, 1200 Larimer St., Denver, CO 80204.

The intracellular free calcium ion concentration is thought to regulate the growth of neurites in several types of cultured neurons. Calcium influx through voltage-sensitive calcium channels increases the intracellular calcium concentration and therefore may influence the rate of neurite growth. In vertebrate neurons, the L, T, and N calcium channel blockers respond differently to heavy metals (Cd**+** and Ni**+**+) and to organic compounds such as verapamil, the dihydropyridines, amiloride, and the aminoglycosides. In primary cultures of neurons from chick embryo brains, L channel blockers, such as Cd**+** (up to 100 μM), nor N channel blockers, such as most aminoglycosides, affect neurite growth. Streptomycin, which has been reported to block N type channels at low concentrations and L type channels at high concentrations, blocks neurite growth only at very high concentrations. Therefore, calcium influx through L type channels appears to promote neurite growth in these neurons.

Supported by grants from NIH and EPA to G. Audesirk.

452.19

**PATCH-CLAMP OF CULTURED CRUSTACEAN PEP TIDICERGIC NEURONS.** D. Myers, R. A. Graf,*, P. Ruben, and J. Corver.

Békésy Laboratory of Neurobiology and Department of Zoology, University of Hawaii, Honolulu, HI 96822.

The neurosecretory cells of the crab (Cardiacosoma ctenica) isolated and cultured in simple, defined media, showed immediate, vigorous outgrowth and retain their biochemical distinctness (Graf, et al., this vol.). We report here that they also retain the voltage-dependent sodium currents expected from in situ studies; Na-current is lacking as cell patch electrodes at the soma were used to voltage-clamp large fluctuations of ca. 5 mV. Action potentials were not observed. Whole-cell recordings made 10-21 days later yielded high frequency ongoing rhythmic firing independent of the electric organ discharges, which was eliminated after resection. Single fibers microdissected from such neuromas were stained with a highly specific polyclonal antibody raised in rabbits against purified Na-channel protein from eel electroplax plasma. Immunolocalization of Na-channel protein that sensory neuron GalTase enzymatic activity could modulate the extracellular milieu during the phase of patterned cell death at which time the catalytic substrate UDPgal might be released to the extracellular milieu.

Supported by NSC Canada and the Canadian Paraplegic Association.

452.20

**THE ORGANIZATION OF MYOSIN IN CULTURED NERVE GROWTH CONES DETECTED BY IMMUNOELECTRON MICROSCOPY IN PC12 CELLS.** C. Bridgewater and M. E. Paine.

Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

A potent minority labeling procedure using rapid freezing, freeze substitution and low temperature embedding in Lowicryl K11M has been adapted for the detection of myosin in cultured rat superior cervical ganglion nerve growth cones. This method allowed conventional immunoelectron microscopy either disturb the structure of growth cones or destroy the antigenicity of myosin. An antiserum specific for the heavy chain of human platelet myosin was used for labeling. Rotary shadowing of purified platelet myosin/antiserum complex showed antibody binding to four electron-dense areas along the tail and head portion of the molecule. Cross-reaction of the antiserum with the rat nerve cells was confirmed by immunoabsorptions and immunodeterminations of frozen, freeze-fractured cultured nerve growth cones. The aggregates were often associated with distinct irregularly shaped areas of increased electron density. Label was not consistently associated with small filaments. The distribution of the myosin label suggests that myosin may play a role in growth cone motility.

(Supported by NIH grant NS05070.)

Laminin facilitates cellular attachment and neurite outgrowth. A 67 kDa laminin receptor protein has been identified, Mcl2 kDa ("tranin"; Smalheiser and Schwartz, 1987) or 110 kDa (Kleinman et al., 1988), which may be a laminin receptor. Antibody, 3070, was raised by immunizing rabbits with the 120 kDa protein. The antiserum demonstrates the major HNK-1-immunoreactive protein of embryonic chick brain (Mr 120,000). An antibody to 3070 immunoreactivity (IR) in aldehyde-fixed chick embryos was examined immunocytochemically along with markers to identify neural crest-derived cells (monoclonal antibody, NC-1) and neuronal precursors (GFAP, IR) and an associated protein (NAPA-73). The earliest localization of 3070-IR, at stage 10, was in the neural tube. At this time, virtually all neural tube cells expressed 3070-IR, but only a small fraction expressed NC-1-IR and these did not co-express 3070-IR. No NC-3070-IR was found in the premigratory neural crest; however, at later stages (19-24) NC-1-immunoreactive cells that co-expressed 3070-IR were found migrating along the aorta, between the mesonephros and within the gut. The neural tube, notochord, dorsal roots and mesonephric tubules also displayed 3070-IR. NT expression was not detected in the 3070-IR cells until stage 26. Many NT-IR cells in the branchial arches failed to express 3070-IR or NC-1-IR. At later stages in the gut 3070-IR was expressed on a subset of NC-1-IR cells and a subset of the 3070-IR cells also expressed GFAP. These observations are consistent with the hypothesis that crest-derived cells do not acquire neural laminin receptors until they begin to differentiate as neurons. Supported by NIH grant # NS 15547.

DEVELOPMENTAL POTENTIAL OF OLIGODENDROCYTES AND ASTROCYTES IN CULTURED EMBRYONIC XENOPUS CILIA.

As part of an extensive analysis of cell proliferation in the developing dentate gyrus, we have determined: 1) the length of the cell cycle, 2) the length of the DNA-synthetic phase (S-phase), and 3) the growth fraction (i.e., the proportion of cells in the hilus that comprise the proliferating population). A cumulative labeling procedure was used. At postnatal day 20, C57BL/6J mice were injected with bromodeoxyuridine (BUDR) at two hour intervals for a total period of 12 hours. Animals were sacrificed at selected intervals, and the brains were processed for immunohistochemistry using an antibody directed against BUDR. Sections from the approximate middle of the septo-temporal axis of the hippocampal formation were selected, and the number of BUDR-labeled and unlabeled cells in the hilus of the dentate gyrus counted. The number of BUDR-labeled cells increased linearly for 9 hours from an initial value of 12% of the total number of cells to a maximum value of 24% of the total. Calculations from these findings indicate that at this age the cell cycle is 18 hours, the S-phase in 9 hours and 24% of the cells in the dentate hilus are part of the proliferating population. In addition, the linear increase in the proportion of BUDR-labeled cells indicates that, at least in terms of the lengths of the cell cycle and the S-phase, the proliferating cells in the dentate hilus at this age comprise a single population.

Supported by NIH Grants NS 23647, AA 0756, AA 06916, DE 07734 and a grant from the UMDNJ Foundation.


We have developed a method for studying cell lineages which uses a retroviral vector, called BAG, to genetically mark embryonic cells in vivo (Price et al., 1987). PRAS 84: 156-160). BAG carries the bacterial β-galactosidase gene which can be detected histochemically, in infected cells, using the substrate X-gal.

We have injected BAG into the cerebral vesicles of rats 13 uteri, and the resulting clones can be isolated in the ventricular cells of the developing cerebral cortex. The virus used is of such a type that infection is a rare event. Analysis of the resultant clones allows us to ask two types of question. Firstly, how different the neural cell types are related; secondly, how do the progeny of a single progenitor cell expand in time and space to contribute to the cortical structure. In preliminary studies we have defined two types of progenitor cell in the developing cortical plate, one that gives rise to astrocytes but not neurons and one that gives rise to neurons and some types of glial cells. Further, we infected embryonic cortical cells in culture and have been able to mark clones similar to the ones we have defined in vivo. This tissue culture system allows us to ask what factors influence the fate of multipotential progenitor cells.

453.11 CHIMERIC ANALYSIS OF LINEAGE RELATIONSHIPS AMONG MOUSE LUMBAR MOTONEURONS. M.W. Vogle, A.W. English, and K. Herrup. Dept. of Human Genetics, Yale University Medical School, New Haven, CT 06510 and Dept. of Anatomy, Emory Univ., Atlanta, GA 30322.

We have analyzed mouse chimeras to address two questions about the role of lineage relationships in the development of the lumbar lateral motor column: 1) Are all lumbar motoneurons descended from a defined group of progenitor cells, and 2) is motoneuron lineage related to motoneuron connectivity, as defined by muscle innervation? We have identified medial ganglionic eminence (MGE) cells by HRP backlabeled motoneurons in β-galactosidase mice, which were embryos carrying BAG vector transducing the tsA58/U91 combination large SV40 T oncogene. At the permissive temperature, one of the derived cell lines proliferates and expresses the T antigen, and small amounts of OC (galactocerebroside), a surface lipid marker for oligodendrocytes and Aβ (surface gangliosides). At the non-permissive temperature, the cell line lose the T antigen expression, stop proliferating, and stain very strongly with GC and Aβ antibodies. In addition, the two main protein components of myelin, MBP (myelin basic protein) and PLP (proteolipid protein) are also expressed.

These results show that a precursor cell line spontaneously differentiates into a mature oligodendrocyte when the immortalizing oncogene is inactivated. The functional or potential use as replacement therapy of these cells will further be explored in transplantation experiments using myelin deficient mutant rodents.


With the aim of producing species-specific monoclonal antibodies that could be used as cell markers in chimeric mice, BALB/c mice were immunized with cell nuclei isolated from Mus caroli brains. A monoclonal antibody (IG) was produced that appears to bind the nuclear membranes of BALB/c nuclei but not BALB/c, mice. Sections of formaldehyde-fixed, polymer wax were embedded in immunohistochemical studies using Vector ABC with HRP. The resulting staining of nuclear membranes gives the appearance of rings, hence, the antigen has been nicknamed "ringo". The epitope recognized by this antibody was present not only in Mus caroli but also in Mus castaneus as well as in all inbred strains of laboratory mice tested including C57BL/6J, C3H/HeN, DBA/2, SWR, AKR and LPT. Since the antibody does not react with the BALB/c (B6) mice, seven CXB (i.e., BALB x B6) recombinant inbred strains were examined and the observed strain distribution pattern matched perfectly with the B6.C-1 allele on Chromosome 16. L6 is located on the distal end of Chromosome 1 (i.e., BALB x B6). The BALB/c nuclei, seven CXB (i.e., BALB x B6) recombinant inbred strains were examined and the observed strain distribution pattern matched perfectly with the B6.C-1 allele on Chromosome 16. L6 is located on the distal end of Chromosome 1 (i.e., BALB x B6). The BALB/c nuclei, seven CXB (i.e., BALB x B6) recombinant inbred strains were examined and the observed strain distribution pattern matched perfectly with the B6.C-1 allele on Chromosome 16. L6 is located on the distal end of Chromosome 1 (i.e., BALB x B6). The BALB/c nuclei, seven CXB recombinant inbred strains were examined and the observed strain distribution pattern matched exactly with the CXB distribution pattern.

In brain sections, the antibody appears to recognize many if not all cell types. The staining is particularly strong in the cerebellum. Preliminary observations suggest rings may be specific to neural tissue and may first become evident sometime after postnatal day 10. We have recently examined a C3H→BALB chimera and mosaicism was evident among cerebellar granule cells and Purkinje cells as well as in the dentate gyrus and hippocampus.

(Supported by NIH Grants NS10156 and EY07017)

453.12 AN ANTIBODY TO RETINAL GANGLION CELLS RECOGNIZES PREMIGRATORY AND MIGRATING CELLS IN THE DEVELOPING CHICK RETINA. Steven C. McConnell and Roxana Severson*. Dept. of Cell Biology & Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

We have developed a monoclonal antibody, R.A.A4, that recognizes retinal ganglion cell axons in the mature retina. Between embryonic days 3 and 9, the R.A.A4 antigen was associated with cells in certain regions of the retina in addition to the optic fiber layer. These R.A.A4 positive cells were of three types: an apolar cell adjacent to the ventricular surface, a bipolar cell that spanned the thickness of the retina and a monopolar cell in the ganglion cell layer. Further analyses revealed that these cells are premigratory and migrating retinal ganglion cells. The expression of the R.A.A4 antigen is the earliest indicator of ganglion cell specification. The existence of R.A.A4 positive apolar cells along the outer surface of the retina suggests that the ganglion cell phenotype is expressed as soon as the cell becomes postmitotic in the ganglion cell layer. In vivo labeling with R.A.A4 and other analyses revealed the R.A.A4 antigen to be a 140kDa cytoplasmic protein in the retina. R.A.A4 is also expressed by many long tract axons in the brain. In the brain, the R.A.A4 antigen was observed to have 7 different molecular weights. Evidence suggests that different cell types express the R.A.A4 antigen with slightly different molecular weights. Finally, the expression of the R.A.A4 antigens in retinal axons appears to be regulated by some factor produced by the optic tectum.
453.13

A simple method for culturing relatively pure populations of radial-like glial cells was devised based on differential adhesion to a non-adhesive substrate. Dissociated embryonic day 18 rat brain regions were plated onto glass coverslips in culture medium containing BME + 10% Nuerom (Collaborative Res.) + pen/strep + dextrose. Due to the initial lack of substrate adhesion, reaggregates were formed which subsequently attached and spread. Regional differences were observed in the size of the reaggregates and their tendency to spread on the substrate, suggesting distinct cell surface properties. Most neurons did not survive after 1-2 days and the remaining cells consisted mainly of undifferentiated neuroepithelial cells or glia as determined by immunoreactivity (IR) with anti-GFAP (Accurate Chem). GFAP-IR cells were observed at E12, large motoneuronal somata with long processes extending as spines of the aggregates. After several days, nuclei were visible within these processes, apparently moving through them to their distal ends. These cells then extended lamellipodia and retracted their proximal process which had been attached to the aggregate, forming a free-aggregating GFAP cell strongly resembling an astrocyte. This culture system may prove useful for investigations of the radial glial lineage.

453.14
CELL MIGRATION AFTER OLFACTORY PLACODE TRANSPLANTATION IN XENOPUS. H. Klim, C.G. Nottegael and P.G. Graziani. Dept. of Biological Sciences, Florida State Univ., Tallahassee, FL 32306-1050.

From stage 23-24 Xenopus laevis we transplanted the olfactory placode to same stage Xenopus borealis in place of the optic vesicle. Differential nuclear staining with quinacrine of the two allowed to distinguish if the cells of the host from those of the donor. Chimeras were observed to stage 51. Usually the transplanted placode fused with the one of the host producing a large olfactory organ. In some animals the donor placode grew independently from the host's. From the transplanted placode a nerve developed and reached the fish's head. Along this nerve many cell with the characteristics of the donor migrated and penetrated the CNS. After stage 45 these cells formed small aggregates. Attempts are under way to determine the specific nature of the migrating cells (glia versus neurons) and their significance and putative contribution. Supported by NSF grant CNS 8617022.

453.15
NOTCH IS REQUIRED FOR CELL DECISIONS IN THE DROSOPHILA RETINA. Ross L. Capes* and Donald F. Ready. Dept. of Biology, Princeton, NJ 08544, and 1Dept. of Biol. Sci. Purdue University, West Lafayette, Ind. 47907.

We have examined the role of the gene Notch during successive stages in the development of Drosophila photoreceptors. Using flies containing the temperature-sensitive allele Notch27, we shifted larvae and pupae to a non-permissive temperature for brief periods and examined the consequences. A diverse array of phenotypic abnormalities were observed, including transformation of one cell type to another. Notch appears to play a role in each retinal cell's choice of fate. Shifting Notch wild-type normally uncommitted cells becoming photoreceptor cells; more posterior ommatidia showed differences in the number of cone cells. Shifts of young Notch mutant pupae affected the number of bristles formed, while later shifts affected pigment cell differentiation. Loss of cell type was associated with the shift in temperature. These results suggest that Notch is required for the specification of cell fate.

453.16
DEVELOPMENT OF CELL-SPECIFIC MARKERS IN CHICK SPINAL GANGLION NEURONS. C.L. Smith, Dept. of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

During development of the dorsal root ganglia, neural crest cells differentiate to form a variety of distinct types of sensory neurons that differ in their sizes, shapes, and histochemical properties. I am investigating the role of peripheral targets in determining the phenotypes of sensory neurons by studying the expression of cell-specific markers by neurons in chick spinal ganglia. Markers that label subsets of sensory neurons in mature embryos have been studied: an antibody to a calcium binding protein (CBP), an monoclonal antibody raised against chick ganglion cells (TGF; see Frank, et al., this volume), antisubstance P (SP), and somatostatin (SRIF). The sensory neurons that express these markers are concentrated in specific regions of the ganglia and differ in average size. Some neurons are both CBP+ and SP+. Immunocytochemical studies in conjunction with retrograde labeling have shown that CBP+ neurons are predominantly cutaneous afferents. SP and SRIF label both cutaneous afferents and muscle afferents. Some sensory neurons express CBP+ or SP+ immunoreactivity at E5 when few neurons have innervated their peripheral targets, suggesting that the early expression of CBP+ and SP+ is not target-dependent. Furthermore, sensory neurons from E5 embryos express CBP+ immunoreactivity when grown in vitro in the absence of their targets. By contrast, CBP+ and SA positive neurons are not observed until E10 or E11, after many sensory neurons have already innervated their targets. Sensory neurons from E5-E10 embryos do not become CBP+ when grown in vitro for 4-6 days. Experiments are now in progress to determine whether sensory neurons grown in vitro become SA+ or SP+ and whether neurons can be induced to express specific markers by growing them with peripheral targets.

Supported by grant NS24470 to C.L.S. and NS24373 to E.Frank.

453.17
A MONOCLONAL ANTIBODY THAT SELECTIVELY LABELS LARGE SENSORY NEURONS IN DEVELOPING CHICK SPINAL GANGLIA. J.E. Frank, H. Tsuruhara*, C.L. Smith and V. Lemmon. Dept. of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Studies of the development of various classes of sensory neurons within dorsal root ganglia (DRGs) would be greatly facilitated by the availability of histological markers for the different neuronal phenotypes. By generating monoclonal antibodies against lumbar DRG antigens, we have isolated a monoclonal antibody, TGF. This antibody stains approximately 50% of neuronal soma in E17 lumbar DRGs. Very few of the small DRG cells that are substance-P-positive stain with TGF, while many of the larger neurons that label with anti-N-acetylaspartylglutamate (anti-NAAG, see Kowalski et al., Brain Res. 406:3971 are also TGF-positive. Axons in the dorsal and ventral columns of the spinal cord are also labeled with TGF. Bundles of stained axons descend from the dorsal column through the dorsal horn to terminate in the substantia gelatina, similar to the projections of muscle sensory neurons. Although the staining pattern is qualitatively similar to that seen with anti-NAAG, which labels neurofilaments, TGF stains fewer axons in the spinal cord and stains some mononuclear rather than larger sensory neurons. The TGF+ by E5, but staining of DRG neuronal soma does not appear until E10 and the number of labeled somata increases until at least E15. At E12, there is a clear division between large ventromedial (VL) and small dorsomedial (DM) DRG cells, and TGF labels neurons primarily in the VL region. At later stages some TGF-positive neurons are found throughout the DRG. The relative number of immunoreactive cells is 4-5 times greater in lumbar than thoracic DRGs; this may be related to the smaller amount of cell death of the VL population at limb versus thoracic levels. Finally, TGF labels both muscle and cutaneous sensory neurons. Neurons in the mesencephalic nucleus of V, known to project largely to muscle spindles, are TGF-positive, as are many large neurons in the cuneate division of the trigeminal ganglion, which project primarily to skin. Similarly, DRG neurons retrogradely labeled with fluorescent latex beads from peripheral nerves are TGF-positive, as are axons in cutaneous nerves in the hindlimb. Supported by NS24373 to E.F.

453.18

In order to examine whether early interactions are necessary for the normal production of a single frog blastomere's progeny, we transplanted single labeled cells of the 16- and 32-cell stages toavel sites in unlabeled hosts. Embryos were fixed at late tailbud stages, serially sectioned, and the distribution of the labeled cells compared to normal fate maps (Moody, 1987, Dev. Biol. 119:560; 122:300). Some transplanted blastomeres took on the characteristics of their new site. For example, v1.1.2 normally becomes a significant contributor of Rohon-Beard neurons, but becomes a significant contributor of motoneurons after transplantation to the dorsal midline. Some blastomeres, however, continued to express their normal neuronal and non-neuronal lineages after transplantation. For example, the 0.1.1 clone produced in v1.2 was transplanted to the ventral vegetal pole was quantitatively indistinguishable from normal. In about a third of cases, and in all experiments in which v1.1.2 was transplanted to the ventral vegetal pole, secondary neural tubes were induced. The 0.1.1 clone was normally distributed in this secondary axis. Supported by Grants NS21158, NS07668, and WIBB Training Grant HD07323.
451.3

CELL LINEAGE AND DETERMINATION III

THURSDAY PM

We investigated whether interactions between cleavage stage blastomeres are necessary for the production of their normal neuronal progeny. Single blastomeres, with or without the third tier of the 32-cell stage (vegetal equatorial cells) were marked with a lineage dye and their anterior neighbor was removed. The dorsal midline cell normally contributes sparsely to the hindbrain and spinal cord, but after ablation it no longer contributed to the CNS in half of the embryos and contributed only a few cells in the rest. The ventral midline cell normally contributed to dorsal hindbrain and spinal cord; after ablation its only neuronal progeny were a few Rohon-Beard neurons. The ventral midline cell normally contributes sparsely to dorsal hindbrain and spinal cord; after ablation it had no neuronal descendants in half of the embryos, and had the normal ones in half of them. The non-neuronal progeny of these cells were not altered by the ablations. Thus, interactions between second and third tier cells significantly influence CNS lineage expression in the frog. Supported by NS23518.

451.4

NEURAL PLASTICITY IN ADULT ANIMALS: ANATOMY AND BEHAVIOR

AMPHETAMINE ENHANCES BEHAVIORAL RECOVERY FROM SENSORY-MOTOR DEFICIT RESULTING FROM INFARCTION OF PRIMARY SOMatosensory CORTEx.

B. Reuter, W.D. Dieterich, P.M. McCabe, B.D. Watson, M.D. Ginsberg, & N. Schneiderman. Depts. of Psychology and Institute for Neurological Sciences, Univ. Texas, Austin, 78712

Previous research in this laboratory has shown that there may be a sensitive period following brain damage during which recovery processes are vulnerable to pharmacological manipulations (Handzschuh et al., 1988). In the present study, we found that there may be a sensitive period following unilateral forelimb sensorimotor cortex (FLS/MTR) lesions during which the contralateral homotopic cortex is especially vulnerable to damage. Rats were given unilateral FLS/MTR lesions with a 24-hour interoperative period (IOPs) between 1 and 14 days. To compare the extent of damage incurred by Lesion 1 to Lesion 2, the volume of the intact cortex contiguous to each lesion was measured. The day IOP was chosen with a 7-day apparent (less remaining cortical volume) in the 2nd lesioned hemisphere. However, the volume of the Lesion 1 cortex did not differ from the Lesion 2 cortex when 2-stage lesions were given with 1 or 14 day IOPs. Furthermore, sensorimotor behavioral asymmetries, as measured by a bilateral tactile stimulation test, paralleled the anatomical asymmetries. The interfaced behavior following 3-stage lesions with 7 day IOPs, the smaller cortical volume in the Lesion 2 hemisphere indicated that the cortex contiguous to the 2nd lesion may be anatomically vulnerable to damage at this stage in recovery. In other words, more extensive damage to the cortex when previous damage was given in the opposite homotopic cortex. However, there appears to be a fixed stage or time period before and after which this vulnerability does not exist. Supported by NH4 grant NS-39946 awarded to T. Schallert.

451.5


Previous studies have shown that Xenopus embryos that are incubated in LiCl during cleavage stages become dorsalized and produce up to twice the normal number of neurons (Handzschuh et al., 1987). We found that the critical period of Li+-mediated dorsalization is between the 32- and 128-cell stage. We investigated whether progressive unilateral EC lesions in neonatal animals caused by changes in neuronal lineages. The fate of individual blastomeres of 16-cell Li+-treated embryos was compared to their fate in the normal embryo (Moody, Dev. Biol. 191:560, 1997). A ventral animal blastomere (V1.1), whose normal neuronal descendants are few and located only in the spinal cord, populated large areas of the forebrain, midbrain, hindbrain and spinal cord. A dorsal animal blastomere (D1.1) produced significantly more progeny in regions of CNS that they normally populate. A dorsal vegetal blastomere (0.2.1) produced small amounts of the CNS, as in the normal embryo. These results indicate that neuronal lineages normally are influenced by events that occur during cleavage stages and that these events are altered by Li+ ions. Supported by HD33234 & NS23518.
Neural Plasticity in Adult Animals: Anatomy and Behavior


In the hippocampus, terminal zones of the perforant path from the entorhinal cortex show high levels of cytochrome oxidase (CO), whereas terminal zones of the commissural and associational fibers show high levels of lactate dehydrogenase (LDH). To study the role of different pathways on post-synaptic levels of energy metabolizing enzymes, we examined the distributions of CO and LDH in rats following lesions of the entorhinal cortex in rats. Animals received unilateral electrolytic lesions of the entorhinal cortex at postnatal periods of 1-3, 4-6, or 8-20 days. These lesions were then prepared for TEM evaluation. In addition to the neuronal surface area (SA) of these cells increases significantly, the number of synapses per unit area also increased. The average size of the synaptic contact area increased by as much as 40% and additional sites occurred on the same spine. We conclude that the increase in the number of synaptic sites precede restoration of synaptic number following acute deafferentation of the hippocampus. These findings indicate that a significant number of the restored sites could arise from remaining synapses through dividing of the contacts and their respective spines. Supported by USPHS NS-20349 & NS-13742 from NINDS.


The number of synaptic sites in the dentate molecular layer begins to be restored by 5 days and returns to near normal by 60 days following lesions to afferent pathways. The source of the new sites remains incompletely understood, involving sprouting of the ipsilateral or contralateral hippocampal and the septal nuclei. A surgical lesion was placed in the entorhinal cortex of the rat at the junction with the subiculum. Morphometry of the heads of spines and synaptic sites at 5 days revealed elongation of the spines so that they partially wrapped the bouton. The average size of the synaptic contact area increased in large spines by as much as 40% and additional sites occurred on the same spine. We conclude that the increases in the size of synaptic sites precede restoration of synaptic number following acute deafferentation of the hippocampus. These findings indicate that a significant number of the restored sites could arise from remaining synapses through dividing of the contacts and their respective spines. Supported by USPHS NS-20349 & NS-13742 from NINDS.

454.7 Entorhinal cortical lesions are accompanied by an alteration in the expression of neural adhesion molecules in the dentate molecular layer. J.S. Suda, D.R. Ramirez*, M. Valbuena* and G.A. Schwarting*. Dept. of Biochemistry, E.K. Shriver Center, Walpole, MA 02154 and Dept. of Psychology, Davidson College, Davidson, NC 28036.

A ganglioside, α-galactosyl, α-fucosyl GM1, which is present in low quantities in rat brain is recognized by a monoclonal antibody, WCC (Suchy et al., Brain Res. 440, 1988). This ganglioside is concentrated in the outer 2/3 of the molecular layer following EC lesions. The change in α-galactosyl, α-fucosyl GM1 expression in the ML corresponds to the time of expansion of commissural-associational fibers following entorhinal cortex lesions. These results may reflect control of oxidative and glycolytic enzyme activity by pathway-specific synaptic input.

Supported by USPHS Grant 1R01 NS-13742 from NINDS.


Unilateral removal of vibrissae (URV) in rats induces an asymmetry in facial scanning in the open-field. This behavioral asymmetry subsides after 1-3 days. Evidence exists for plastic changes in the crossed nigrostriatal projection subsequent to URV. In this experiment we determined the time-course of this neural plasticity and compared it to that of the behavioral recovery. After URV for 1-3, 4-6, or 8-20 days rats were injected with horseradish peroxidase into the stria terminalis (CPU) ipsilateral or contralateral to URV. Retrogradely labeled cells were counted in both substantia nigra, URV/1-3 rats had a greater crossed projection to the CPU ipsilateral than contralateral to URV. This asymmetry was reversed in URV/4-6 rats and reduced in URV/8-20 rats. The latter group had a greater uncrossed projection to the CPU contralateral to URV. Thus, these results indicate a similar time-course for the neural plasticity as for the behavioral recovery after URV.

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454.9 RAPID FORMATION OF NEW DOUBLE SYNAPSES IN RAT SUPRAOPTIC NUCLEUS (SON) IN RESPONSE TO INTERRUPTION OF SUBFORNICAL ORGAN EFFERENT PROJECTIONS. M.L. Weiss, C.D. Tweedle, F. Marzbey*, B.K. Modney*, and G.J. Hatton, Michigan State University, Neuroscience Program, East Lansing, MI 48824-1117.

Previous work with transmission electron microscopy (TEM) has shown that damage to the subfornical organ (SFO) produces degenerating terminals within the SON (Brain Res. 275: 365, 1983). In an attempt to track and quantify these changes, we made use of the SFO or its efferent pathway (SFOX), and following a survival period of 24 hr, were prepared for TEM evaluation. In addition to finding occasional degenerating fibers, we found significant increase in the percentage of cells contacted by terminals which formed synapses upon two adjacent somata in the SON (soma-somatic "double" synapses, 9 cells). An additional increase in the percentage of soma contacted by single synapses was found. Since the neuronal surface area (SA) of these cells increases significantly with dehydration, the percentage measures listed above were multiplied by SA to determine the number of cells contacted by each synapse. The number of synapses was found to increase in dehydration relative to controls. Direct membrane apposition and the formation of new double synapses may play an important role in the coordinated activity of these neurons during periods of chronic hormone release. Supported by NIH grant NS019140.

454.10 DEHYDRATION INDUCED CHANGES IN RAT SUPRAOPTIC NEURONAL CELL SIZE IS ACCOMPANIED BY ABSOLUTE INCREASES IN MULTIPLE SYNAPSES, NEURONAL MEMBRANE APPPOSITION AND GLIAL CONTACT: B.K. Modney* & G.J. Hatton (SPON: A.K. Salm) Neuroscience Program, Department of Psychology, Michigan State University, East Lansing, MI 48824-1202.

Extensive alterations in rat SON morphology occur during chronic dehydration. Changes occurred specifically in the nuclei of neuron cell bodies (n=6) and dehydrated rats (10 days drinking 2% saline) revealed significantly increased percentages of cell membrane contacted by adjacent cells or dendrites and multiple synapses (a single terminal forming two synaptic contacts). A significant decrease in the percentage of somatic membrane contacted by single synapses was found. No change in percentage contacted by glial processes was found. Since the neuronal surface area (SA) of these cells increases significantly with dehydration, the percentage measures listed above were multiplied by SA to determine the number of cells contacted by each synapse. The number of synapses was found to increase in dehydration relative to controls. Direct membrane apposition and the formation of new double synapses may play an important role in the coordinated activity of these neurons during periods of chronic hormone release. Supported by NIH grant NS019140.
The impact of the hippocampus by sprays of peripheral sympathetic fibers following medial septal lesions provides a unique model for studying collateral sprouting in the CNS. The sympathetic sprouts are mainly restricted to the hilus and granule cell and supragranular layers of the dentate gyrus, and in the stratum lucidum and stratum radiatum of CA3 of the hippocampal formation (HF) where they replaced the lesioned septohippocampal neurons (Czuchry, K. A., Brothers, C. M., & Oakley, J. B., Neurosci. 66:778; 1997). The aim of the present experiment is to study the biochemical changes associated with the cellular events which takes place over the course of a septal lesion. The model used was the HF of the adult rat, since it has been demonstrated that the HF is invaded by sympathetic fibers from the superior cervical ganglion and noradrenergic afferents to the nucleus, has been studied by retrograde tract-tracing methods, and by eliminating contribution of peripheral vascular CA for more than 3 days after implantation. The HF is invaded by sympathetic fibers from the superior cervical ganglion. The cellular events which takes place when, as the result of a medial septal lesion in the rat, synaptic inflow from subiculum reaches area 29c of the hippocampus. In the present experiment is to study the biochemical changes associated with the cellular events which takes place over the course of a septal lesion in the rat, synaptic inflow from subiculum reaches area 29c of the hippocampus. Thus, adult nerve cut does not alter the area or size of the HF, whereas the enzymatic activities of the HF are depressed compared to control levels in the unlesioned rat. In the snorx sympathetics, the HF is invaded by sympathetic fibers from the superior cervical ganglion and noradrenergic afferents to the nucleus, has been studied by retrograde tract-tracing methods, and by eliminating contribution of peripheral vascular CA for more than 3 days after implantation. The HF is invaded by sympathetic fibers from the superior cervical ganglion. The cellular events which takes place when, as the result of a medial septal lesion in the rat, synaptic inflow from subiculum reaches area 29c of the hippocampus. Thus, adult nerve cut does not alter the area or size of the HF, whereas the enzymatic activities of the HF are depressed compared to control levels in the unlesioned rat. In the snorx sympathetics, the HF is invaded by sympathetic fibers from the superior cervical ganglion and noradrenergic afferents to the nucleus, has been studied by retrograde tract-tracing methods, and by eliminating contribution of peripheral vascular CA for more than 3 days after implantation.

Rats raised in complex environments (EC) have larger neurons with more synapses in the occipital cortex than rats raised individually (IC) or in isolation (IC) (e.g. Turner & Greenough, Brain Res., 1985). These results suggested that synaptic communication might be more intense in ECs than ICs. Astrocytes have been shown to be involved in ionic and neurotransmitter regulation and so may also influence the efficacy of synaptic transmission. Preliminary examination of astrocytes in the cortex of EC, SC and IC rats showed that astrocyte nuclei were larger in ECs than ICs. This implied that astrocytic arborization might also be greater in ECs than ICs. This hypothesis was tested by using indirect immunocytochemistry to label astrocytic processes with an anti-GRF and then estimating the surface area (SA) of these processes by the stereotaxic technique of vertical sections. This study showed that ECs have a 17% greater SA of astrocytic processes than ICs; that the density of astrocytes is 12% lower in ECs than ICs; and that the mean surface area per astrocyte is 27% greater in ECs than ICs. SA values were similar in all measures. Supported by PHS 2T32GM07143 and Ml 35321.

COMPLEX EXPERIENCE INDUCES CAPILLARIES IN VISUAL CORTEX OF ADULT RATS. J.E. Black, A.M. Sirevaag, and W.T. Greenough, College of Medicine, Deps of Psychology and Cell & Structural Biology, and Neural & Behavioral Biology Program, University of Illinois, Urbana 61801.

Young rats reared with other rats and toys generate many new capillary branches within 30 days (Black, et al., Neurosci Lett. 83: 351), but angiogenesis in old rats provided complex experience seems to be impaired (Issacs, et al., Soc Neur, 12: 1579). The present study examines angiogenesis in 6-3 month-old rats either given complex experience (EC) or kept in isolation (IC) for periods of 10, 30, or 60 days and then perfused. All vessels are easily seen in toluidine-stained 2 um sections of visual cortex. The visual cortex of EC rats was significantly thicker than that of IC rats after 10, 30, and 60 days. This corresponds to previous estimates of greater synaptogenesis in EC rats (Hwang & Greenough, Soc Neur, 12: 1284) which presumably spread apart existing blood vessels. Capillary spacing was nearly identical for EC and IC rats after 10 and 30 days, however, indicating that EC rats made new capillaries. EC rats also had a smaller mean capillary diameter than IC rats after 10 days, consistent with the presence of many immature and small capillaries. These findings suggest that angiogenesis in the mature cerebral cortex begins within 10 days of complex experience and requires at least 60 days to reach completion. Supported by NMD, 35321.

BEHAVIORAL PHARMACOLOGY: ACETYLCOLINE

EFFECTS OF SCOCOLAMINE ON A DELAYED SPATIAL MATCHING TASK IN RHESUS MONKEYS. R.F. Genovese and T.F. Elsmore*. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

We investigated the effects of scopolamine (1-12 ug/kg) on a novel spatial learning task where three rhesus monkeys were trained to interact with images on a CRT. Eight circles, arranged in a circular pattern, were presented on the screen and the one circle (chosen randomly) contained a distinctive feature. Touching the feature cleared the display for .01, 4, or 16 s. A choice screen consisting of the eight circles, with the featured stimulus, was then presented. Touching the circle that had previously contained the feature, produced a food pellet and an exploration activity peaks at this age. 12 littermate pairs of 30 day old male Long-Evans hooded rats were randomly assigned to differential housing for 4 days.

COMPLEX HOUSING FOR ONE LITTERMATE CONSISTED OF A LARGE CAGE CONTAINING OTHER RATS AND AN ASSORTMENT OF TOYS (EC). RC rats spent one hour per day in a play cage while toys in the home cage were replaced. Littermates of the RC animals were housed individually (IC) for 4 days. Basilar dendrites of layer III pyramidal cells were drawn from Golgi impregnated sections. Total dendritic length and number of branches at each order of bifurcation were averaged. RC rats exceeded IC rats in total dendritic length. These results indicate that the effects of exposure to a complex environment are manifest rapidly in weanling rats and are compatible with the hypothesis suggested by previous results (Turner & Greenough, Brain Research, 1985) that the effects of a complex environment on neuronal morphology involve synaptogenesis in response to environmental stimulation. Supported by NMD 35321.


Previous studies have demonstrated that 30 days of housing in a complex environment alters dendritic morphology of various neuron types in the visual cortex. This study assessed these changes after a much briefer exposure to complex housing. Weanling rats were used, as their exploration activity peaks at this age. 12 littermate pairs of 30 day old male Long-Evans hooded rats were randomly assigned to differential housing for 4 days.

This study investigated the effects of scopolamine, a muscarinic receptor agonist, on a visual recognition task in two adult male rhesus monkeys. The monkeys were initially trained to perform on a serial probe recognition (SPR) task with a variable list length design that closely parallels tests given to humans: a simultaneous discrimination, which does not involve a memory component, showed no significant change from control days. The decline in visual recognition ability in these monkeys closely resembles the decline in memory observed in humans with cholinergic insufficiencies, i.e., Alzheimer's disease. This data suggests a strong cholinergic component in this type of visual retention task. This paradigm provides an excellent opportunity to explore new and current therapeutic approaches in memory disorders by enhancement of cholinergic transmission.
455.3 EFFECTS OF DAILY REPEATED SOMAN EXPOSURE ON TRACKING PERFORMANCE OF JUVENILE AND ADULT RATS. S. M. Bell, V. C. Pellis, and S. C. Pellis*. Dept. of Psychology, University of Florida, Gainesville, FL 32611.

We estimated the daily dose of soman, an organophosphate nerve agent, to be 4.0 mg/kg/day in order to produce a small but reliably detectable decrement in the performance by rhesus monkeys of a well-learned compensatory tracking task, the Primate Equilibrium Platform (PEP) task. Monkeys were tested on a daily basis with a fixed dose of soman (1.64% of the threshold dose of ED50) for a performance decrement on or before the 5th daily exposure was found to be 0.97 ± 0.05 (SEM) mg/kg/day, about 40% of the single-dose acute ED50. Behavioral effects of daily repeated soman exposure are much more variable than the effects of a single, acute exposure. Blood ChE inhibition is a poor predictor of behavioral effects.


Rats were trained to reach through a rectangular hole and exert downward forces on a force transducer. As long as the force on the transducer was maintained above a threshold, the rats were able to drink from a dipper of sweetened condensed milk. After a two-week period of 10-min daily practice on this task, the rats were divided into 4 groups and treated with ip, injections of vehicle (V) or haloperidol (Hal, 0.04, 0.08, 0.16 mg/kg) for 17 consecutive days. On the 15th drug day, the 4 groups received an ip. injection of atropine sulfate (AS, 5mg/kg) in addition to Hal. The dependent variables were time-on-task (the amount of time the rat's forelimb force remained above 20 g) and the integrated variance ("power") of force oscillations having frequencies within a 5-to-23 hertz bandwidth. Hal alone increased time-on-task and increased the variance of force oscillations. When AS was given along with Hal, the disruptive effects of Hal on performance were significantly attenuated. These data suggest that the behavioral deficits engendered by low doses of haloperidol in rats are analogous to neuroleptic-induced parkinsonian symptoms in human beings. (Supported, in part, by NIH 43629.)


The specificity of glucose interactions with cholinergic function and the role of GLU interactions with cholinergic function and the role of GLU interactions in paradoxical sleep. Additionally, GLU augments the severity of hyperactivity, suggesting that the locomotor effects of GLU are mediated by central or peripheral GLU actions.

455.6 CHOLINERGIC-DOPAMINERGIC INTERACTIONS IN RADIAL ARM MAZE PERFORMANCE ROLE OF NICOTINIC CHOLINERGIC SYSTEMS. R. D. McGuck, E. D. Levin, and L. L. Butcher. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Interference with either muscarinic-cholinergic or dopaminergic function impairs radial-arm maze performance. Paradigmatically, the amnestic effect of scopolamine is significantly reduced by simultaneous dopaminergic blockade, suggesting that cognitive function is dependent upon a balance between these neurotransmitter systems. Since central nicotinic receptors are involved in cognitive functioning, and because blockade of nicotinic receptors impairs maze performance, this experiment examined the interaction of nicotinic-cholinergic and dopaminergic systems.

Rats were trained on an 8-arm radial maze. Drug testing began after asymptotic levels of performance were reached. All rats received saline, mecamylamine (2.5-10 mg/kg), haloperidol (0.04, 0.08 mg/kg), or a combination of mecamylamine and haloperidol (2.5 and 0.4 mg/kg, respectively) in a counterbalanced order. Maze performance was significantly impaired by the higher doses of mecamylamine or haloperidol. Interestingly, although the lower doses of mecamylamine and haloperidol did not affect maze performance, their combination caused a significant decrease in performance. Taken together, these results reinforce the importance of dopaminergic and nicotinic-cholinergic systems in maze performance and indicate a significant interaction of these systems. That subthreshold doses of nicotinic-cholinergic and dopaminergic antagonists summate to interfere with maze performance suggests that the circuitry underlying this behavior contains both a nicotinic and dopaminergic synapse. Finally, these data illustrate the differential involvement of muscarinic and nicotinic systems in learning. (NIH grant NS-10928 to L.L.B.)

455.7 NICOTINE ENHANCES PLAYFUL ATTACK IN THE PLAY-FIGHTING BY JUVENILE RATS. R. M. Bell, V. C. Pellis and R. L. Lennon*. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Systemic (S.C.) injection of nicotine acid produced a dose dependent increase in play-fighting in juvenile rats (30-40 days old). By using 'pinning' (ie. one animal standing over the other, or partner) as the measure of play-fighting, the increase was not significant over vehicle controls at 0.125 mg/kg but was so at 0.5 mg/kg. However, pinning involved both attack and defense, so that an increase in play-fighting could arise from an increase in either attack rate or defense rate. Therefore, attack (ie., lunging at nape) and defense (ie., pinning to suppress attack) of each rat was measured separately for each of the seven subjects involved.

After 24h isolation sibling rats were reunited, 15 min after one animal received an appropriate injection and their behavior was videotaped under red-light for 10 min. Attack in the injected rats increased significantly with increasing dose, whereas defense remained unchanged. Neither the rate of attack or defense increased in the non-injected rat. The rats did not respond to stimulation by electrical frequencies of attack, not of defense.


The consequence of a schedule of nicotine treatment to rats that is said to increase the specific binding of [3H]ACh or [3H]nicotine to nicotinic cholinergic sites in the cerebral cortex was tested as the sequel to the acquisition of platform location in a Morris water maze. Rats were trained for 5 days with saline or a low dose (0.2 mg/kg) of nicotine and then followed each day's trials. Previous reports that this treatment increases locomotor activity in the first minutes following injection were confirmed. Nicotine treatment did not alter the consolidation of the spatial task because the rate of task acquisition, measured as latency to find the platform, was not different between the two groups. After a trials-without treatment, at a time when nicotine receptor up-regulation in the cortex is said still to be present, the animals were retested for their memory of the task and found to be performed worse. In an attempt to augment cholinergic function, tetrahydrocannabinol (THC) was admistered 1 hr pre-test to all animals; a significant decrease in latency to find the platform from the first trial to the last trial was observed in the group treated with nicotine. (Supported by MRC Canada.)
The responses of rats to nicotine solutions were examined using the taste reactivity (TR) test and 2-bottle 24 hr preference test. In Experiment 1, non-deprived rats were administered intracranial infusions of water, 1 µg/ml, 5 µg/ml, 25 µg/ml, and 100 µg/ml nicotine, and TR responses were videotaped and analyzed. Nicotine, up to 50 µg/ml, elicited ingestive TR responses. Ingestive responses significantly increased and aversive TR responses significantly increased in response to 100 µg/ml nicotine. Next, 2-bottle preferences for water vs. 1 µg/ml, 5 µg/ml, and 0 µg/ml (water control group) were measured in 3 groups of naive rats. After 16 days of exposure, rats showed a significant preference for 1 µg/ml nicotine. The preference for 5 µg/ml nicotine significantly increased during the experiment, but it remained less than that for 1 µg/ml and 0 µg/ml nicotine. Last, TR responses elicited by 1 µg/ml and 5 µg/ml nicotine were measured in the rats having had the 2-bottle experience. Rats showing a 2-bottle preference for the 1 µg/ml nicotine showed significantly more ingestive TR responses to 1 µg/ml and 5 µg/ml nicotine that did the control rats. These data show that prolonged voluntary access to nicotine results in an increased preference for nicotine; i.e., modifies the rats' immediate oral/gustatory reactivity to nicotine.

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456.3 STRIATE AND EXTRASTRIATE ASYMMETRIES IN JUVENILE AND ADULT HUMAN BRAINS. M.-C. de Lacoste, C.D. Kim, L. N. Smith*, D. J. Woodward, Dept. Cell Bio., U. T. Southwestern Medical Ctr., Dallas, TX 75235

We have previously reported striking occipital regional volumetric asymmetries in a large number of primate species at all phylogenetic levels (de Lacoste et al., 1988). The aim of this study was to determine if, in juvenile and adult humans, the "gross" asymmetry reflects an underlying asymmetry in primary visual (striate,ST), extrastriate (XT), or both ST and XT cortex.

The CSCAN utility of CARP (Computer Aided Reconstruction Package) was used for semiautomated delineations of 1) the external and internal borders of cortical gray and 2) the boundaries between ST and XT (per techniques described in [Smith, de Lacoste et al 1987]). CARP was used also for areas and volumes.

Preliminary results indicate that both ST and XT are highly asymmetrical in human adults (ST: T=1222; ST: 11-20%). In fact, occipital regional volumetric asymmetries favor opposite hemispheres. Our data further suggest that juvenile and adult males exhibit greater ST and XT asymmetries than female counterparts. In sum, our results indicate that both primary and associational areas are organized in the left and right hemispheres of the human brain. Future studies will assess volumetric asymmetries in subdivisions of ST i.e. visual association cortex. Supported by HD21711 to MCL and the Biological Humanics Foundation.


Ongoing studies in this laboratory have explored the application of computer graphics and image processing techniques to three-dimensional reconstruction from planimetric measurements of neuroanatomical form. The CARP (Computer Aided Reconstruction Package) contains software modules which allow manual tracing, morphometry, computer microscopy, C-142D autoradiography and three-dimensional reconstruction and image synthesis. A sophisticated hierarchical, anatomical database provides storage for various forms of data including video images, graphical representations of tissue sections and three-dimensional reconstruction models. Experience has also revealed a need for the ability to attach arbitrary text descriptions to graphical and image data which represent associations between the stored data and information from other sources. For example, boundaries denoting cortical regions may have appended anatomical names, descriptors or lists of bibliographic references. A screen-oriented menu allows users to manipulate a LISP data structure called the property table which associates property-value pairs with a node in the CARP database. Once created, the property list serves both as cross references with other information sources and as targets for searching paradigms in CARP database operations. Current applications include retrieving complex descriptions to regions of striate and extrastriate visual cortex in primate brain. Support from DARPA, AA9W1 and the Biological Humanics Foundation to DJW, HD21711 to MCL and SBIR NO1-NS25321 to WKS (Biographies, Inc.).

456.5 HEMISPHERIC ASYMMETRIES IN LOGICAL CATEGORIES: CONVERGENT EVIDENCE FROM NORMAL AND SCHIZOPHRENIC SUBJECTS. D. W. Zaidel and K. Frederick. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

In a previous study (D.W. Zaidel, Cognitive Neuropsych, 4:321-332, 1987), hemispheric asymmetry was found for retrieval of information about natural categories from long-term semantic memory. The purpose of the present study was to determine whether any asymmetries are present for logical categories. Both normal and patients with complete section of the forebrain commissures are studied. The task consisted of deciding whether or not numerals are ODD or EVEN. Stimulus numerals represented the following bins: 5, CQ, M0CCO, or M0CQCC0. They were flashed in the left or right visual fields and the answer was indicated with a key-press. Both accuracy and reaction latency were recorded. Results showed a high accuracy rate in both visual fields. Analysis of latencies revealed significant interactions for field X parity and for field X bin (e.g. S3, M0CQ)


Interhemispheric electrocorticographic asymmetries have been demonstrated during the processing of affective sensory information in the human using auditory and visual evoked potential (VEP) recordings. The present study extends these findings in conjunction with brain electrical activity mapping (BEAM, Caldwell - model 8400) of VEPs was utilized to characterize interhemispheric responses to auditory stimulus. Pattern reversal VEPs were recorded from 20 right-handed subjects (10 males/10 females) using the standard 10-20 electrode layout. Subjects were and attended to music (non-verbal) and continuous speech (verbal) stimuli.

Examination of BEAM scans and a cross-correlation analysis generated from the recording sites revealed no reproducible hemispheric asymmetries associated with any of the conditions. VEPs recorded under all conditions were normal for latency and amplitude. No reproducible asymmetries were noted on separate analysis of F7-F8, T3-T4, C3-C4, O1-O2. The inability to detect previously reported electrocorticographic asymmetries may reflect the modality of the probe or the complex asynchronous nature of the auditory stimulus.
456.9 INTERHEMISPHERIC RELATIONSHIPS

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Association cortices, or asymmetry in the development of sulci in the cortical region did not reveal side differences in these monkey species. Significant greater mean length (p<0.05; ANOVA) revealed. Evidence for functional asymmetry were taken on the cortex of formalin-fixed brains of New World monkey species. We found other measurements on the temporoparietal area which usually suggest asymmetry in Homo brains. In accord with this finding, results of several studies showed that sustained neglect was induced in both PCm groups by SPIRO. This was replicated in an objective study in which judges indicated that emotions can be turned on by sustained unilateral facial muscle contractions. They are consistent with the view that the right hemisphere is implicated in negative emotional experiences and that the left hemisphere has a different but not easily specified function. They provide a method for studying hemispheric specialization for emotional experience and have implications for relations between emotion and cognition.

456.11 DICHOTIC LISTENING IN RELATION TO SEVERITY OF CLOSED HEAD INJURY. H. S. Levin, W. M. Higginbotham, D. H. Williams, F. G. Amato, and N. M. Eisenberg. The University of Texas Medical Branch, Galveston, TX 77550

Eighty-two right-handed patients who sustained a closed head injury (CHI) of varying severity were characterized according to the level of the deepest lesion visualized by magnetic resonance imaging (MRI). Patients with no lesions or extradural and/or cortical lesions were grouped together (n=31) while patients with lesions extending into the subcortical white matter or deeper comprised another group (n=51). Thirteen right-handed controls were also examined. All patients and controls were screened for hearing loss and tested on dichotic listening using a computer synthesized tape which consisted of six consonant-vowel nonsense syllable pairs presented simultaneously via earphones. The subjects were instructed to repeat the syllables presented on each trial. A laterality index reflected the preference for reporting the syllables heard by the left or right ears. Nonparametric analysis of variance indicated that patients sustaining moderate to severe CHI showed a significantly greater right ear advantage in dichotic listening performance than controls. Furthermore, among the moderate to severe injuries, patients with parenchymal lesions extending into the subcortical white matter showed a greater right ear advantage as compared to controls, whereas the laterality index of patients with lesions which were higher on the neuraxis did not differ from results in controls.

456.12 ANATOMICAL BRAIN ASYMMETRIES IN MONKEYS

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Measures of symmetric and asymmetric brain asymmetry were taken on the cortex of formalin-fixed brain specimens from two Old World and three New World monkey species. Our findings are consistent with previous reports in that our results indicate that the right hemisphere is superior in the discrimination of inverted faces. While the interpretation of the inversion deficit is controversial, the finding that the data from monkeys is similar to that from people suggests that similar mechanisms of laterality are being examined in both species.

456.13 DICHOTIC LISTENING IN RELATION TO SEVERITY OF CLOSED HEAD INJURY. H. S. Levin, W. M. Higginbotham, D. H. Williams, F. G. Amato, and N. M. Eisenberg. The University of Texas Medical Branch, Galveston, TX 77550

Eighty-two right-handed patients who sustained a closed head injury (CHI) of varying severity were characterized according to the level of the deepest lesion visualized by magnetic resonance imaging (MRI). Patients with no lesions or extradural and/or cortical lesions were grouped together (n=31) while patients with lesions extending into the subcortical white matter or deeper comprised another group (n=51). Thirteen right-handed controls were also examined. All patients and controls were screened for hearing loss and tested on dichotic listening using a computer synthesized tape which consisted of six consonant-vowel nonsense syllable pairs presented simultaneously via earphones. The subjects were instructed to repeat the syllables presented on each trial. A laterality index reflected the preference for reporting the syllables heard by the left or right ears. Nonparametric analysis of variance indicated that patients sustaining moderate to severe CHI showed a significantly greater right ear advantage in dichotic listening performance than controls. Furthermore, among the moderate to severe injuries, patients with parenchymal lesions extending into the subcortical white matter showed a greater right ear advantage as compared to controls, whereas the laterality index of patients with lesions which were higher on the neuraxis did not differ from results in controls.

456.14 LATERALITY IN MONKEYS DISCRIMINATING INVERTED FACES. B. A. Vermeire and C. R. Hamilton. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The right hemisphere of human beings is usually found to be superior to the left in the recognition of faces. The present experiment, 16 split-brain monkeys who had previously learned to discriminate upright from inverted faces. While the interpretation of the inversion deficit is controversial, the finding that the data from monkeys is similar to that from people suggests that similar mechanisms of laterality are being examined in both species.

Supported by MH-34770.
Recent prenatal studies have revealed left-right hemisphere asymmetries of neurotransmitter levels, dopamine receptor binding, glucose metabolism and morphological features of the corpus striatum. Using a novel paradigm, the present study reveals that activation of the right striatum has a qualitatively different effect on behavior of rats than activation of the left striatum. To produce a relatively selective activation of the right and left striatum, the model of Ungerstedt was employed, where injection of apomorphine in a rat with a 6-hydroxydopamine lesion of the substantia nigra activates, preferentially, the postsynaptic dopamine receptors on the side of the lesion. By modifying the paradigm of Pisa and Szechtman (Neurosci. Letters, 44:41, 1986), we assessed whether the direction of swimming in a large circular swimming pool was influenced by drugs. We report here that with activation of the right side of the pool, rats were attracted to the edge of a pool and swam along it in a direction that was the reverse of their preferred direction in the middle of the pool. In contrast, with activation of the left striatum, rats showed little attraction to the edge and no consistent directional preference when swimming along it. These findings suggest that the right striatum controls contralateral orientation and the left striatum controls bilateral orientation.

**MHC TYPE AND LATERALIZATION: DEGREE OF ASYMMETRY FOR HANDHELDNESS AND SWIMMING ROTATION IN CONGENIC MICE**

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H-2 types were differentially distributed in lines of mice selectively bred for degree of handedness. Whereas G0 comprised six H-2 types, strongly lateralized G24 B6 line mice were predominantly H-2d, and weakly lateralized I/LJ mice were mostly H-2b (R. L. Collins, G. A. Carlson & J. H. Nadeau, Society for Neuroscience Abstracts 11:861, 1985). To further relate MHC type to degree of asymmetry, handedness and swimming rotation were examined in two panels: H-2 congenic mice (n = 334): BALB/cBy (H-2d) and C.B6-H-2d; and C57Bl/6By (H-2b) and B6.C-H-2b/a (BW19), B6.C-H-2b/a/BW19), B6.C-H-2b/a/B6 (BW19). Mice were tested for handedness in the unbiased or ‘U-world food-reaching task’ and then twice later in worlds biased opposite to their handedness—’U-R-R’ or ‘U-L-L’ (R. L. Collins, Science 187:181-184, 1975). Data measures across tests were expressed as paw entries consistent with original hand preference. Swimming rotation for 2 minutes in a circular water maze was observed for three days. Swim measures were summed preferentially rotations/total rotations.

Handedness tests yielded partial support for the research hypothesis: (BALB > C.B6) and female (BW19, BW19> B6. However, male B6 and C.B6 mice did not show the desired pattern. For degree of swimming there was no Sex × Genotype interaction: (BW19, BW19) > B6. Although BALB and C.B6 mice swam hardly at all. Female mice of all groups tended to swim counterclockwise. For males, the direction was balanced.

Overall results support the hypothesis that H-2d mice are more strongly lateralized as H-2b mice. Either the H-2 complex itself exerts pleiotropic influence on degree of lateralization, or genes affecting lateralization reside at loci located near the H-2 complex on Chromosome 17. Results are consistent with aspects of the Geschwind hypothesis (N. Geschwind & P. Behan, PNAS 79:5097-5100).
During development, axonal muscles affiliated with MHD express an impaired ability to differentiate to the metabo-
lic-enzymic profile characteristic of normal (N) glyco-
ytic muscle. This determination if this (N) phenotype is programmed within D myogenic cells by day 2 in ovo. D somitic mesoderm was transplanted to replace N brachial somitic mesoderm due to the limited ability of embryos to hatch following somite transplantation and the fact that metabolic differentiation is completed ex ovo. A two-step procedure was necessary. Thus, the protocol derived from D somitic mesoderm grafted at day 2 in ovo to an N host (step 1) was then transplanted at day 16 in ovo to a newly hatched N host (step 2). As a control, a similar two-step protocol was performed between N donors and hosts. The degree of metabolic differentiation achieved was assessed histochemically (phosphorylase, SSH reactions) during a postoperative period of 5-16 weeks ex ovo. Results indicate that the D phenotype of impaired metabolic differentiation was expressed by genotypically D muscles whereas geno-
typically N muscles differentiated normally. Thus, we con-
clude that specific D phenotypes, not overtly expressed until development ex ovo, are programed by day 2 in ovo. Furthermore, these are influenced by extrageneric fac-
tors associated with a N environment during development in ovo and ex ovo. (MDAC, NEHC supported).

**507.5**


Embryonic cortical grafts surviving in cavities made in motor/sensory cortex of adult rats contain nearly normal numbers of pyramidal neurons (J. Neurosci. 7: 3002, 1987). However, very few retro-
grade-labeled pyramidal neurons are seen in similar transplants from fetal host cortex. However, is exogenous (Exp. Neurol. 99: 156, 1987).

Juvenile rats (30-60 days) received focal frontal cortex transplants in motor/sensory cortex as previously described. A month later sections were processed for immunocytochemistry. A monoclonal antibody (12/18) was used to stain all neurons. A monoclonal to neurofilament protein (NFP) selectively labeled pyramidal cortical neurons of layers I-VI. The number of neurons in transplants and normal cortex was similar. All pyramidal neurons but perhaps a smaller number was less than in normal cortex. This number, how-
ever, was much greater than the number of pyramidal neu-
rons that established connections with host thalamic cells in previous work. Some grafted pyramidal neurons were aligned in rows, but their processes were not perpendicularly to the oral surface as in normal cortical laminae.

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either host brain or transplant vessels during graft revascularization, 2-3 week old rats had pieces of fetal neocortex (E18-21) or mature autonomic ganglia placed in either ventricle IV or directly into the parietal cortex. After postoperative survival times of 8 hours to 1 week, 6u paraffin sections were processed for PAP immunocytochemistry using antibodies to FN or LN. There was a significant increase in FN staining in brain vessels adjacent to parenchymal grafts at 24-48 hours, when graft revascularization occurs. Neocortical transplant vasculature also stained more intensely for FN during the first week. Growing CNS parenchymal vessels, whether immature or regenerating, express more FN than uninjured, mature brain vessels, while LN appears unchanged. Supported by Am. Heart Assoc. and NS-17468.

457.9


Mesopallium from day E-16 fetuses was implanted into cavities produced by hippocampal aspiration in rats. The implants established interfaces with the host brain parenchyma and lesion surfaces. SEM revealed that, rather than the normal variation in ventricular surface features (dense cells and/or microvilli), the implant surface is a matrix of intertwoven processes and cells. The cells appear to give rise to the matrix of neaurmphic processes. Smooth oval cell bodies can be found individually and in small groups interspersed across the matrix. These cells resemble cultured ganglion cells (neurons) or oligodendroglia. Other cells are flattened and have course surfaces and resemble cells in brain aggregate culture. There are sparsely distributed regions with typical appearing cells. Clumps of cilia can also be observed arising from shafts of some neurmphic processes. In addition, there are regions of microvilli similar to regions found on the normal ventricular surface. The transplants appeared to have surface features similar to those described in CNS cavities formed in response to pathological conditions such as amyotrophic lateral sclerosis (Supported by USPHS Grant #5RO1NS070 to MLW).
457.13


Adrenal medulla is commonly used as a source of catecholaminergic tissue for neural transplantation. We thought it might be advantageous to culture medullary tissue (transplantation in medium containing NGF or dexamethasone (dex), since NGF, which facilitates neurite outgrowth, may shift the proportion of the 3 major catecholamines (CA) noradrenaline (NE), epinephrine (E) and dopamine (DA); and since dex enhances cell survival. Therefore, we examined whether culturing medullary tissue in medium containing NGF, NGF plus dex, or dex, either, affected the proportion of CA relative to fresh tissue. Medullary tissue was dissected from adrenal cortex and cut into approximately 1 mm³ pieces. 4-5 pieces were frozen immediately (fresh). The rest were cultured with fresh media. Total protein was measured using the Bradford method. Results: 1) CA contents in fresh tissue, expressed as CA/total protein were: E, 4.55 x 10⁻²; NE, 1.98 x 10⁻³; and DA, 4.6 x 10⁻³. 2) Following 3.5 or 12-14 days of culture, E and NE decreased by 50-90%, although NGF tended to prevent the loss of E and NE. 3) DA content was similar in fresh and cultured tissue. Therefore, it appears that culturing medullary tissue results in a decline in E and NE but not DA. It is possible that culturing medullary tissue in the presence of NGF and/or dex affected the proportion of CA relative to fresh tissue.

457.15


It has been shown that transplantation of fetal basal forebrain and central nucleus of amygdala disrupt retention of passive avoidance learning. Moreover, it has been reported that amygdaledenervated animals showed spontaneous recovery of a previously lost learned task. In this work we demonstrated that fetal brain transplants can accelerate the spontaneous recovery of memory in amygdaledenervated animals. Male Wistar rats were randomly divided in five groups. Four groups sustained large amygdaloid lesions and one remained as an unoperated control group. All animals were trained to avoid foot-shock in a shuttle box, follow by five retention trials. After training two groups of lesioned rats received homotopic fetal brain transplants in the amygdala and the other two remained lesioned control groups (Lxx). The 7A and Lxx animals were assigned in two pairs of groups. One pair of groups were retrained one month later and the other two months later. The results showed that all animals tested after two months recovered the ability to learn the task. However, those grafted animals retrained one month later significantly improved the retention of the learned task as compared with the lesioned group. These results suggest that there are spontaneous recovery in amygdaledenervated rats in a passive avoidance learning task, and this recovery can be hastened by grafted tissue.

457.17


Recently we have demonstrated that the fetal brain transplants produced recovery of taste aversion learning in adult rats with grafted cortical basal ganglia which retinalized otherwise dead brain tissue. The results were obtained by using transplants from 32-55 days gestational age fetal rat brain as well as the same tissue, but decorticated. In this work we investigated the influence of target and age of donor tissue on parameters of graft survival. F-344 rats (n=27) were lesioned bilaterally with ibotenate in the NBM. Three weeks post-surgery rats received either, cortical injections of E-16 fetal cell suspensions (n=4), or NBM injections of E-16 suspensions (n=5), or NBM injections of E-16 suspensions (n=5). Nine lesioned rats served as inoculated control and the other two months later. The results showed that all animals tested after two months recovered the ability to learn the task. However, those grafted animals retrained one month later significantly improved the retention of the learned task as compared with the lesioned group. These results suggest that there are spontaneous recovery in amygdaledenervated rats in a passive avoidance learning task, and this recovery can be hastened by the presence of grafted tissue.

457.14

AN EXAMINATION OF THE ABILITY OF CORTICAL TRANSPLANTS TO REVERSE MEMORY IMPAIRMENTS INDUCED IN ADULT RATS BY LESIONS OF THE NUCLEUS BASALIS IN RATS. A.C. Santucci, V. Haroutunian, R. Gluck and K.L. Davis. Bronx VAMC & Mt. Sinai School of Medicine, New York, NY 10468.

Although cortical transplants have been reported to reduce memory impairments produced by nucleus basalis of Meynert (nBM) lesions, little information is available concerning those transplant parameters that optimize recovery. Accordingly, 7-10 days after bilateral nBM lesioning, rats received cell suspensions of fetal tissue (centrally) to either 0 (nBM), 2 (2TR) or 4 (4TR) frontal cortical locations. Sham-lesioned animals (SH) without transplants were used as a memory task that required subjects to find a water-filled receptacle among an array of 6x5 receptacles was employed. Animals received one trial/day and trials 6 errors-to-criterion served as dependent measures. Relative to the SH group, nBM and 2TR subjects required more trials and committed more memories errors before attaining criterion (p<.05). Finally, the 4TR group did not differ from nBM animals (p>.20). Experiments in progress examine the generality of this transplant-induced alleviation of lesion-induced memory impairments. Histological and neurochemical analyses will be conducted at the completion of behavioral testing.
457.19

Cerebellar suspensions prepared from normal mice embryos (E12) were grafted to the cerebellum of 45 day-old Purkinje cell degeneration (pcD) mutant mice which, by that age, have lost virtually all of their Purkinje cells (Po). Graft development was monitored at various times by the use of antibodies against two proteins localized specifically to Po in normal cerebellum: 28-KDa Ca-binding protein (CalB) and 7.6-KDa FEP-19 (gifts of M.R. Cello and J.L. Morgan respectively). CalB immunoreactive cells were detected in the host molecular layer 9 days after transplantation (corresponding age of grafted Po neonatal) and persisted throughout the survival time allowed (3 months). FEP-19 immunoreactivity was evident as early as 5 days after transplantation (corresponding age of grafted Po E17) and persisted throughout the survival time allowed as well. The morphological development of immunoreactive Po appeared very similar with both antibodies. Following grafting, somatic thorns were present at 9 days and elaborate dendritic arbors from 17 days on. These results indicate the ability of grafted Po to retain features of normal chemical differentiation.

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458.1
QUANTIFICATION OF REACHING MOVEMENTS IN SUBJECTS WITH SPASTICITY. S. E currently attending Dept. of Physical Therapy, Boston University, Boston, MA 02215.

Kinematic data were used to quantitatively describe components of reaching movement (normal vs. spasticity). Movements were classified as normal, spastic or ataxic. Dependent variables included displacement of the hand and number of accelerations/decelerations during reaching to target. The WATSMART three dimensional motion analysis system and video were used for data collection.

Dependent variables included displacement of the hand and number of accelerations/decelerations during reaching to target. A single acceleration/deceleration combination was a movement unit (L.Fetters & J. Todd, J.Motor Behav., 19:2, 1987).

Reachers and children with spastic quadriplegia included significantly more movement units than children with normal motor abilities. In addition, the number of movement units decreased following exercise with the spastic subjects.

The analysis of movement units (stop/start action) is a viable method for characterizing reaching movements and for assessing change in subjects with motor disability.

457.20

Macaque embryos were individually raised to age 6 months in large cages built into one wall of a 4 foot by 4 foot cage to provide access to it but not tactile contact. Two deprivation conditions were tested (Cond 1 and Cond 2) and both were tested in the same cage and with respect to partial social isolation. They differed in the amount of somatosensori-motor opportunity available to the subjects. In Cond 1, the Cond 2 chamber was empty, whereas Cond 3 contained ladders, and a trapeze. Four monkeys from each of the conditions were overlaid with four colony-reared (Cond CR) monkeys. The neuroanatomical changes resulting from these conditions were assessed by counting dendritic spines on the apical shafts of layer IIb pyramidal cells in VI cortex, and by counting spines in Golgi-stained tissue. Layer IIb pyramidal cells with somas of medium size were selected for analysis, in which a sample of 10 such neurons was selected from each cortical region and the density of apical dendritic spines determined. A total of 191 cells were analyzed. The basic dendritic branches of these same neurons were traced, and dendritic branching complexity assessed in order to determine the effect of the deprivation conditions on dendritic morphology and branching. We found that apical dendritic spine density was significantly reduced with both Cond 1 and Cond 2 (which did not differ from each other). This occurred in both VI and SI cortex, but did not in VI cortex, the region used as a control for a generalized brain effect. Branching complexity on the same pyramidal neurons was reduced only in MI cortex of Cond 2. These results show spine density, a more direct measure of neuronal connectivity, to be the more sensitive measure of early environmental deprivation. Also, the enriched environment provided by Cond 3 relative to Cond 2 offset the effect of partial social isolation such that both morphometric measures were comparable to Cond CR monkeys.

458.2
DEPRIVED EARLY SOMATOMOTOR-MOTOR EXPERIENCE IN STUMPFALLEN MONKEYS: FREEZING, MOTOR DEPRESSION, AND EXTRAPYRAMIDAL SYMPTOMS. MEASURED BY CORTEX PERIUMBILICAL (CPO) DOPAMINE AND DENDRITIC BRANCHING OF LAYER IIIB PYRAMIDAL CELLS. T. Pasik and P. Pasik. Dept. Biomedical Sciences and Psychology Dept., University of California, Riverside, CA 92501.

Several protocols were tested in which important somatosensori-motor opportunity available during development in that the cond 2 chamber was empty, whereas cond 3 contained ladders, and a trapeze. Four monkeys from each of the conditions were overlaid with four colony-reared (cond CR) monkeys. The neuroanatomical changes resulting from these conditions were assessed by counting dendritic spines on the apical shafts of layer IIb pyramidal cells in VI cortex, and by counting spines in Golgi-stained tissue. Layer IIb pyramidal cells with somas of medium size were selected for analysis, in which a sample of 10 such neurons was selected from each cortical region and the density of apical dendritic spines determined. A total of 191 cells were analyzed. The basic dendritic branches of these same neurons were traced, and dendritic branching complexity assessed in order to determine the effect of the deprivation conditions on dendritic morphology and branching. We found that apical dendritic spine density was significantly reduced with both Cond 1 and Cond 2 (which did not differ from each other). This occurred in both VI and SI cortex, but did not in VI cortex, the region used as a control for a generalized brain effect. Branching complexity on the same pyramidal neurons was reduced only in MI cortex of Cond 2. These results show spine density, a more direct measure of neuronal connectivity, to be the more sensitive measure of early environmental deprivation. Also, the enriched environment provided by Cond 3 relative to Cond 2 offset the effect of partial social isolation such that both morphometric measures were comparable to Cond CR monkeys.

458.3

The pallidum of 20 rhesus monkeys, newborn to 4 months in age, was examined in Golgi material and ultrastucturally. All neuronal types seen in the adult are found at birth. The most common large fusiform cell shows initial signs of immaturity: blunt processes and dendritic dilatations at bifurcation points, growth cones, filopodia and filiform processes. By 4 months, they appear fully mature and may be used in surgical therapy. Affixes emerging from the ventral surface do not show yet clusters of varicosities at 2 weeks. At this age, plexus of fine beaded fibers cover large extensions of the nucleus. These fibers were rarely seen with the light microscope at time in all ages . The afferent radial fibers of striatal origin are observed from birth. They form bundles only after 8 weeks, and the density of their climbing branches varies over time. Afferents emerging from the ventral surface do not show yet clusters of varicosities at 2 weeks. At this age, the plexus of fine beaded fibers cover large extensions of the nucleus. These fibers were rarely seen with the light microscope.

Ultrastructurally, the basic neuropil organization is present at birth although with immature features: incomplete covering of the dendrites with axonal boutons, low level of myelination of axonal fibers, presence of growth cones, filopodia and filiform processes. Initially, most dendrites show large varicosities and immature features: incomplete covering of the dendrites with axonal boutons, low level of myelination of axonal fibers, presence of growth cones, filopodia and filiform processes. By 4 months, they appear mature at all ages examined. Afferents entering the monkey pallidum during the first 4 postnatal months, and suggest a faster rate of maturation than that of the neocortex, probably as a reflection of its neurophilic origin. Supported by NIH Grants NS4-21533, NS-18657 and NS-11631.

458.4

Treatment with amphetamine increases the rate of recovery of beam-walking in rats after a unilateral suction-ablation lesion of the sensory-motor cortex. While investigating this effect, we noted that several rats which had received amphetamine unexpectedly did not recover while several other rats which had not were completely recovered. Affixes emerging from the ventral surface do not show yet clusters of varicosities at 2 weeks. At this age, plexus of fine beaded fibers cover large extensions of the nucleus. These fibers were rarely seen with the light microscope.

Two protocols were tested in which important somatosensori-motor opportunity available during development in that the cond 2 chamber was empty, whereas cond 3 contained ladders, and a trapeze. Four monkeys from each of the conditions were overlaid with four colony-reared (cond CR) monkeys. The neuroanatomical changes resulting from these conditions were assessed by counting dendritic spines on the apical shafts of layer IIb pyramidal cells in VI cortex, and by counting spines in Golgi-stained tissue. Layer IIb pyramidal cells with somas of medium size were selected for analysis, in which a sample of 10 such neurons was selected from each cortical region and the density of apical dendritic spines determined. A total of 191 cells were analyzed. The basic dendritic branches of these same neurons were traced, and dendritic branching complexity assessed in order to determine the effect of the deprivation conditions on dendritic morphology and branching. We found that apical dendritic spine density was significantly reduced with both Cond 1 and Cond 2 (which did not differ from each other). This occurred in both VI and SI cortex, but did not in VI cortex, the region used as a control for a generalized brain effect. Branching complexity on the same pyramidal neurons was reduced only in MI cortex of Cond 2. These results show spine density, a more direct measure of neuronal connectivity, to be the more sensitive measure of early environmental deprivation. Also, the enriched environment provided by Cond 3 relative to Cond 2 offset the effect of partial social isolation such that both morphometric measures were comparable to Cond CR monkeys.

These data show that differences in the motor recoveries of these selected groups of rats did not correlate with lesion volume, depth, or hippocampal damage. The lack of correlation of recovery with lesion size is consistent with observations of recovery of function in man.

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458.5 ELECTRICALLY INDUCED LOCOMOTION IN THE DEAFFERENTED CAT, USING VENTRAL ROOT ISOLATION OR SPINAL CORD PREPARATION. Y. Atsuta*, F. Garcia-Rill and R.D. Skinner. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Recently we described the ability to induce adult-like, coordinated stepping following electrical stimulation of the hindlimb in hindlimb-attached, in situ brainstem-spinal cord preparation (Atsuta et al, Anat. Rec. 220, 1988). These findings suggest the presence at birth of supraspinal systems capable of controlling stepping in adult-like patterns. The present study employed the hindlimb-attached in situ brainstem-spinal cord preparation from 0-4 day old rats maintained in oxygenated artificial CSF. After establishing the control threshold-frequency relationship, the dorsal roots to the attached limbs were severed and the procedure repeated. No changes in threshold or qualitative differences in the locomotor pattern were observed after dorsal root deafferentation. The mean frequency of alternation induced before deafferentation was 0.37 ± 0.6 Hz and, after deafferentation, was 0.43 ± 0.12 Hz. In some cases, remarkably fast alternation was seen only after deafferentation. There was an average increase of 13% (not statistically significant) in the frequency induced at the same electrical threshold following deafferentation. These results suggest that, 1) the supraspinal control of spinal oscillators is not dependent on afferent input, and 2) afferent input, in some instances, may limit the maximal frequency of alternation of the limbs. Supported by USPHS grant NS 20246.


Reinnervating motoneurones specify the force output of denervated muscle fibers after complete and partial nerve injuries to restore the normal size relationships, but whether they fully change properties of the muscle fibers it supplies is uncertain. In correla-tive electrophysiological and quantitative histochemical studies of mouse muscles in rat triceps surae and rat tibialis anterior muscles we have found that discrimination of fiber types according to contractile speed, "sag" and fatigability becomes far less reliable after reinnervation. An increased proportion of units become intermediate in fatiguability and the normal property of fast units to sag during unfused tetani is not a reliable criterion for fast units. Histochemically, muscle fibers are readily classified into types whose proportions are not changed by reinnervation but whose distribution in the muscle is clustered rather than widely distributed. Initial observations that the metabolic properties of muscle fibers within a motor unit are significantly more heterogeneous in character than they are normally may account for loss of sharper distinctions between motor unit types after reinnervation. Supported by the MDA, MRC and AFMR.

458.8 BILATERAL PROJECTIONS FROM MOTOR CORTEX TO THE NUCLEUS OF Darkschewitsch AFTER MONOTAL OR ADULT HEMISPHERECTOMY. R.L. Sutton and Z.R. Villelaiba*. Deps. of Psychiatry and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

The descending cortical pathways to the n. of Darkschewitsch (ND) were examined in cats with neonatal (N) and adult (A) left cerebral hemispherectomy (HEMI) after injection of [125I]-bungarotoxin binding sites per mg protein was used in homogenate preparations of cat triceps surae muscles, 3 weeks to 8 months after (1) spinal cord isolation and deafferentation or (2) ventral root isolation and deafferentation. Numbers of binding sites were significantly elevated in all muscles at 3 weeks after surgery. However they returned to normal 8 months in the muscles with intact motor innervation (1) in contrast to the denervated muscles (2) in which the numbers remained elevated. TMS recordings showed that all muscles fibrillated within the first month after surgery but this activity ceased in the muscles with intact innervation by 2 months. These results provide evidence that acetylcholine receptors are down-regulated in inactive muscles with intact motor innervation suggesting that intact but silenced motoneurones can regulate extrafunctional receptor density in inactive muscles.

Supported by NIH and NEI Grant (5R01AG02562-03).

458.9 SPARED DESCENDING PATHWAYS CONtribute TO THE RECOVERY OF BIPEDAL BUT NONMOPEDAL MOTOR FUNCTION AFTER HEMISECTION IN THE ADULT CAT. M.E. McBride and M.E. Goldberger. Medical College of Pennsylvania, Phila., PA 19129.

Recovery of locomotion and reflex activity following hemisection occurs in precise stages, suggesting that different pathways mediate recovery of nonmonopedal responses. In fact a significant decrease in thresholds were measured for monopedal hopping and placing on the chronically side accompanied by a progressive recovery of postural reflexes on the acute side. Kinematic analysis of conditioned overground and treadmill locomotion documented a recovery of bipedal activity, but recovery of accurate placement during precise locomotion did not recover. These results suggest that contralateral systems are not responsible for recovery of monopedal responses but do contribute to complex bipedal activity necessary for accurate limb placement. Supported by NIH grants NS24707, NS0629, & NSF grant NS00541.

458.10 ENVIRONMENTAL ENRICHMENT DURING GROWTH AFFECTED BODY SIZE AND SHAPE OF GERRIDS. MaryLou Cheal, Cheryl A. Alonzi* and Mary H. Marzke*. Deps. Psychology & Anthropology, Arizona State University, Tempe, AZ 85287.

Environmental enrichment facilitated rapid growth in adolescent gerbils (Cheal, 1984). In order to maximize potential differences in body length during earlier growth, gerbils (n = 35) were born and group reared in large cages (35 X 45 X 66 cm) with locomotor incentives, or in small rat cages (18 X 20 X 25 cm). Body weight, body length, body segment lengths, and ratios of segment length/body length were measured on alternate days from birth to 60 day and at 8 mo of age. From 2-4 wk, when locomotion increases dramatically and pups are weaned, enriched gerbils had longer body segment lengths than controls. Between 7-9 wk, body segment lengths and forelimb length/body length ratios were greater in the enriched gerbils. By 8 mo, significant differences unrelated to allometric effects remained only in that length. It was concluded that absolute size differences due to environmental enrichment may be short-term, but proportions may be permanently affected.
458.11
POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RAT SPINOTRACHTUM, W.C. Broaddus, J.J. Hollerman, and J.C. Dinner, Department of Neurology, Neuroscience and Physiology, Stony Brook University, Stony Brook, NY 11794-8424.

The present study investigates the development of dopaminergic systems in the postnatal rat brain. A sectioning and immunohistochemistry method was used to identify dopaminergic neurons and fibers. The results showed that dopaminergic neurons and fibers develop in a caudal-to-rostral and ventral-to-dorsal order. The development of the nigrostriatal system was found to occur earlier than the mesolimbic system.

458.12
EFFECTS OF HYPOXIC-PHOSPHORYLATION ON POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEM IN THE RAT SPINOTRACHTUM, W.C. Broaddus, R. Rossignol, and J.C. Dinner, Department of Neurology, Neuroscience and Physiology, Stony Brook University, Stony Brook, NY 11794-8424.

The present study investigates the effects of hypoxic-phosphorylation on the development of dopaminergic systems in the postnatal rat brain. A sectioning and immunohistochemistry method was used to identify dopaminergic neurons and fibers. The results showed that hypoxic-phosphorylation affects the development of the nigrostriatal system, with a delay in the appearance of dopaminergic neurons and fibers in the striatum.

458.13
THE ONTOGENY OF NAAG-LIKE IMMUNOREACTIVITY IN THE RAT SPINOTRACHTUM AND ITS RELATION TO SPINAL SENSORY AXONS, M. H. Neale, K. R. McLaughlin, M. E. Houghton, W. M. Houghton, and F. M. Sweetman, Dept. of Biology, Georgetown University, Washington, D.C. 20057 and Dept. of Neurology, University of Miami School of Medicine, Miami, Fl 33136.

The present study investigates the development and localization of NAAG-like immunoreactivity in the rat spinal cord. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of NAAG-like immunoreactivity. The results showed that NAAG-like immunoreactivity is present in the spinal cord and dorsal root ganglia, and is associated with spinal sensory axons.

458.14

The present study investigates the number and size of axons immunolocalizing the masticatory motor axon component of the rat facial nerve. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of masticatory motor axons in the facial nerve. The results showed that the number and size of masticatory motor axons are present in the facial nerve and are associated with the masticatory muscles.

458.15

The present study investigates the rehabilitation of masticatory vibrissae after facial nerve section and repair in rams. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of masticatory motor axons in the facial nerve. The results showed that masticatory motor axons are present in the facial nerve and are associated with the masticatory muscles.

458.16
NIGRAL DA CELL RECRUITMENT AS A COMPENSATORY MECHANISM, J.R. Hollerman and A.J. Grace, University of Pittsburgh, Department of Behavioral Neuroscience, Pittsburgh, PA 15260.

The present study investigates the role of nigral DA cell recruitment as a compensatory mechanism. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of DA cells in the substantia nigra. The results showed that DA cell recruitment is a compensatory mechanism that allows for the maintenance of normal DA cell function in the presence of DA cell loss.

458.31

The present study investigates the mechanism of nigral DA cell recruitment as a compensatory mechanism. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of DA cells in the substantia nigra. The results showed that DA cell recruitment is a compensatory mechanism that allows for the maintenance of normal DA cell function in the presence of DA cell loss.

458.5
THE EFFECTS OF 6-HYDROXYDOPAMINE ON POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RATS, E. D. Mroczek, R. J. Rossignol, and J. C. Dinner, Dept. of Neurology, Neuroscience and Physiology, Stony Brook University, Stony Brook, NY 11794-8424.

The present study investigates the effects of 6-hydroxydopamine on postnatal development of dopaminergic systems in rats. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of dopaminergic neurons and fibers. The results showed that 6-hydroxydopamine has a delayed effect on the development of the nigrostriatal system, with a delay in the appearance of dopaminergic neurons and fibers in the striatum.

458.7
POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RATS, E. D. Mroczek, R. J. Rossignol, and J. C. Dinner, Dept. of Neurology, Neuroscience and Physiology, Stony Brook University, Stony Brook, NY 11794-8424.

The present study investigates the postnatal development of dopaminergic systems in rats. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of dopaminergic neurons and fibers. The results showed that dopaminergic neurons and fibers develop in a caudal-to-rostral and ventral-to-dorsal order. The development of the nigrostriatal system was found to occur earlier than the mesolimbic system.

458.9
POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RATS, E. D. Mroczek, R. J. Rossignol, and J. C. Dinner, Dept. of Neurology, Neuroscience and Physiology, Stony Brook University, Stony Brook, NY 11794-8424.

The present study investigates the postnatal development of dopaminergic systems in rats. Immunohistochemistry and computer-assisted quantitative methods were used to determine the distribution of dopaminergic neurons and fibers. The results showed that dopaminergic neurons and fibers develop in a caudal-to-rostral and ventral-to-dorsal order. The development of the nigrostriatal system was found to occur earlier than the mesolimbic system.

It has been generally thought that conversion from sucking to chewing depends on a peripheral events, i.e., excitation of the jaw. However, this cannot be the case in guinea pigs which are born with mature orofacial structures including complete permanent dentition. The conversion from sucking to chewing is considered to result from change in central mechanisms. We investigated this possibility in ketamine-anaesthetized guinea pigs. To produce rhythmical jaw movements, intracranial microstimulation was used. The following results were obtained: 1) Suckling was only induced in neonates, chewing only in adults. These were driven by different groups of cortico-bulbar neurons through rhythm generator in the brain stem. 2) Cortico-bulbar neurons which elicit sucking were located among the agranular cortex, while those which elicit chewing were located in the granular area of the granular cortex. The latter was newly formed during postnatal development. 3) During development, neurons of the agranular cortex lost their projection to the brain stem and became to activate the newly formed chewing area in the brain stem. It is suggested that transitions in cortico-bulbar projections form the bases of the change in jaw movements.


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The purpose of this study was to determine the changes in size and SDH activity of diaphragm fibers after denervation or blockade of phrenic nerve activity. In 7 adult rabbits were studied: 1) Controls; 2) Denervated (DNV); or 3) TTX-treated. Phrenic nerve activity blocked by TTX. After 30 days, muscle sections were cut and analyzed histologically. Fiber size was also determined using the IPS. SDH activity of type I fibers was reduced in both DNV and TTX animals. SDH activity of type II fibers also decreased in DNV animals, but increased in the TTX group. In both DNV and TTX groups, the size of type I fibers increased. The size of type II fibers also increased in TTX animals, but decreased following denervation. We conclude that differences in the adaptive responses of muscle fibers following denervation or inactivation are related to the persistence of neurotrophic influences in the TTX group.


Previous reports have documented the uptake of injected tritiated proline by glia and its subsequent translocation along axonal pathways. We report evidence of transcellular labeling of glia with secondary transport of labeled amino acids during the development of corticopontine projections in cats. A mixture of tritiated proline and leucine was injected into the primary somatosensory area of the cerebellar cortex. The animals were sacrificed and their brains fixed and processed for autoradiography. Cortical axons in the ipsilateral pontine nuclei (PN) were heavily labeled. Silver grains were present over the neocortex and outlined the somatic profiles of PN neurons. Silver grains were observed only in neonatal cats. Supported: NIH Grant NS20227.


Partial epilepsy, the most common form of seizure disorder, is often uncontrolled by current antiepileptic medication, leaving researchers to concentrate on the need for more effective and less toxic antiepileptic drugs. We have previously shown that ip and intrafocal injections of aminophylline (ADO) receptor agonists can reduce the severity of kindled seizures. IP administration of these compounds can also result in an increase in the strength of postictal EEG and behavioral depression, and a decrease in postictal spiking frequency. The ADO antagonist caffeine (CAF) can block these effects even when administered simultaneously, increases the number of ADO A1 binding sites. In the present experiment we reasoned that prolonged exposure to CAF would increase ADO receptor density and mimic ADO agonist effects on kindled seizures. Sixteen Long Evans rats were implanted with bipolar stimulating/recording electrodes in right amygdala. Once daily electrical stimulation was then delivered until animals were fully kindled. Eight rats were then injected ip with 35mg/kg/day CAF for 1 wk, followed by 35mg/kg/day for 2 wks. The remaining rats served as saline injected controls. Data following the final CAF injection rats were retested for seizure severity and postictal measures. Animals treated with CAF displayed less postictal spiking (p<.05) and extended postictal depression (p<.05) as compared to controls. No differences in seizure severity between groups were observed. These data support the involvement of brain adenosine system in the postictal state. (Supported by NIH Grant RR-08167, and a W.S. Neuroscience Prgm Fellowship to C.L.).
EPILEPSY: SUBSTANIA NIGRA AND AMYGDALA

AUTORADIOGRAPHIC LOCALIZATION OF REDUCED ALPHA 2
effect of cocaine and hypothermia upon kindling in rats. As a
canine use can be combined in some individuals with acute convulsion
toxicity, or possible seizures (lethargy, convulsions)
so it was suggested to us that kindling in the SN and amygdala
through a variety of stimuli (Post, 1976), we began a comparative study of
cocaine, hypothermia, and kindling in rats.

PHASE 1: Four male and four female siblings each of eight litters,
were divided into groups receiving 1) hyperthermia (5°C water bath for four minutes) 2) cocaine (10mg/kg) plus hypothermia 3) "normothermia" (3°C water bath for four minutes, placebo plus "normothermia". Same sex, weight matched, siblings pairs from drug and nondrug groups were run concurrently. All treatments were administered daily from 45 days of age for 16 trials. 15/18 group 2 animals convulsed early (trials 1-10) and of these 8 died. No other animals displayed convulsions through trial 16. PHASE II: Twenty rats, 10 drug and 10 controls, were run at 21 days of age for 16 trials in a massed trial procedure. The results were as follows:

- NI: 12/20 convulsed, latency 24 sec, N=12, p<0.05
- NP: 10/20 convulsed, latency 17 sec, N=12, p<0.05

We hypothesize that the immature SN may exhibit altered pharmacologic response to compounds which are anti-convulsant in adult rats; related to differences in local receptor populations or nigral efferent pathways.

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Bilateral infusions of AP7 (22.5 pg) into the SN of adult rats increased seizure latency compared to saline infused controls [AP7 707 ± 16 sec (x ± S.E.), N=12, Sal 509 ± 18 sec, N=12, p<0.05].

Injections of 2-amino-7-phosphono-heptanoic acid (AP7 - an NMDA agonist) into the SN of adult and 16 day old rats on FD-induced seizures.

Bilateral infusions of the same concentration of AP7 into the SN of 16 day old rat pups did not alter seizure latency compared with saline infused controls [AP7 497 ± 12 sec, N=11, Sal 509 ± 18 sec, N=11, p>0.05].

We hypothesize that the immature SN may exhibit altered pharmacologic response to compounds which are anti-convulsant in adult rats; related to differences in local receptor populations or nigral efferent pathways.

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Norepinephrine (NE) powerfully inhibits the development of kindling, an effect expected through alpha 2 receptors on target NE neurons. McIntyre and Wong (1986) demonstrated reduced alpha 2 mediated inhibition of epileptiform bursts in amygdala-pyramidal cell slices of kindled rats. We therefore hypothesized that kindling produces a reduction of alpha 2 receptor and/or receptor coupled response. Quantitative radioisotopic analysis of alpha 2 receptor were performed to test this idea. Male Sprague-Dawley rats were kindled by amphetamine sensitization and sacrificed 24 hours after 3 days of training. Ten microns brain sections from kindled and control rats were frozen mounted onto microscope slides, incubated with 2.5 nM [3H]-para(3,5)-aminoclonidine ([3H-PAC) at 25°C for 45 minutes in the absence and presence of 100 M phentolamine, rilmen, dield, dexamphetamine, exposed for 8 weeks, and viewed by computer-assisted microdensitometry. Measurements of specific [3H]-PAC binding disclosed a significant reduction in the central nucleus of amygdala (26%, p<0.05) and pyriform cortex (24%, p<0.05), but not basal, median, pre-pyramidal area or adjacent neocortex. The present data extend previous findings from this and other studies (Stanford and Jeffery 1985; Chen et al. 1986), demonstrating a significant reduction of synaptic binding to alpha 2 receptors in kindled rats. This reduction may be a major molecular event underlying the attenuation of NE's inhibitory effect and might thereby contribute to the development of kindling.
EPILEPSY: SUBSTANTIA NIGRA AND AMYGDALA


Sound-induced seizure susceptibility of the genetically epilepsy-prone rat (GEPR) has been partially attributed to an innate defect in central noradrenergic and serotonergic function. This study was conducted to determine whether epinephrine, a possible central nervous system neurotransmitter, has a role in seizure regulation in these animals. The assay of epinephrine concentration in hypothalamus of developing and adult GEPRs as well as in peripheral organs of adult GEPRs was established using high performance liquid chromatography. A significant elevation in its levels in the hypothalamus, heart and kidney of adult GEPRs was not found to be associated with the seizure intensity. Moreover, the difference in hypothalamic epinephrine content of the developing GEPRs did not correlate with their seizure intensity.

LY87130, an inhibitor of the epinephrine synthesizing enzyme, FNMT, reduced seizure intensity, perhaps by increasing extracellular epinephrine. LY51641, a monoamine oxidase inhibitor which elevates intracellular epinephrine, did not alter seizure indices. Results of the present study thus indicate that epinephrine may not play a major role in the seizure regulation in GEPRs.


The effects of small intrathalamic injections of the GABA agonist muscimol on pentyleenetetrazol (PTE) seizure thresholds and spontaneous behavior were determined and compared with the effects of injections of the GABA transaminase inhibitor, gammavinylgABA (GVG). Muscimol injections in the dorsal midline thalamus facilitated the activity of the paraventricular, paratemporal, interanteromedial and intermediodorsal nuclei, as well as the central medullary nucleus facilitating PTE myoclonic tonic seizures; and also produce a sleep-like state. The more posterior of these midline injection sites also inhibited tonic seizures. Injections lateral, dorsal or ventral to this midline region had much less effect on both seizures and behavior. In contrast, GVG injections in the anterior medial thalamus elevate the threshold for all PTE seizure types but have little behavioral effect. These results demonstrate that inhibition of discrete thalamic regions with muscimol can modify seizure expression but inhibition of larger thalamic regions, as with the GVG injections, is required for a general anticonvulsant effect. Supported by NIH Grant NS-11566 and the Sealy Neuropharmacology Research Fellowship.


Temporal lobe epilepsy, the most prevalent form of adult seizure disorder, is also the most resistant to treatment. The efficacy of electrical stimulation as a therapeutic alternative to ablative surgery in suppressing temporal lobe seizures was investigated in this study.

Male Long Evans rats were implanted ipsilaterally with bipolar electrodes in the basolateral amygdala and the dorsal hippocampus. Stimulation (200 µA, 2 second train of 50 Hz, 2 ms bipolar pulses) in the amygdala until three consecutive stage 5 seizures were exhibited. Once kindled, suppressant stimulation was applied to the dorsal hippocampus. Preliminary results indicate that suppressant stimulation delivered to the dorsal hippocampus simultaneously with amygdaloid kindling stimulation can significantly reduce seizure severity from stage 5 to stage 1 in 80% of the trials.

Afterdischarge duration recorded from the amygdala and hippocampus was reduced during 80% of the trials. Suppressant stimulation delivered to the dorsal hippocampus 4.8 seconds after seizure onset potentiated seizure severity and amygdaloid afterdischarge duration.


Recently published data suggest that a restricted site in the deep prefrontal cortex (PFC) may be a critical site for epileptogenesis in rat brain. To test the applicability of this idea, in the kindling model, we electrically kindled different groups of rats in or near the DPC, or in pyriform cortex or amygdala (AM). In different areas of rats we first kindled the DPC and then tested for transfer kindling to the ipsilateral DPC, amygdala, or other areas. We then kindled the AM and tested for transfer kindling to the ipsilateral AM, and 3) injected carbocablol into the DPC using a syringe pump and implanted chemitrodes.

The rates of kindling in DPC (8.1 days, immediately scattered), in AM (5.3 days, scattered and narrow), and in pyriform cortex (8.1 days) were indistinguishable, and were marginally faster (P=0.051-0.08) than kindling in AM (11.0). The DPC lesion group kindled after 8.7 days, which did not differ from the AM group. Picosomal quantities of carbocablol failed to evoke epileptic spiking, and monoamines were usually evoked spiking. Transfer kindling therefore attaining DPC, which is 2.8 days, with the stimulus similarity in kindling between DPC and AM. Thus, DPC kindles readily at a rate that is similar to that of surrounding forebrain tissue, and the integrity of DPC is not necessary for normal AM kindling. Supported by Grants from NSERC to D.P.C. and M.E.C.


Stimulation of either the substantia innominata (SI) or prefrontal cortex (PFX) has been shown to induce generalized seizures. Both of these areas have widely dispersed efferents to each other. In this study, we tested whether SI and PFX in generalized seizures was studied by lesioning each area respectively and stimulating the other to halothane anaesthetized rat. Since PFX has a lower seizure threshold than SI, it was postulated that SI seizures might spread via efferents to PFX and that lesioning of PFX might reduce seizure severity.

After a cortical penicillin spike focus was established in the ipsilateral hemisphere, unilateral bipolar stimulation of SI (0.2-0.6mA, 2.0ms, 400Hz) was carried out to induce generalized electrographic seizures. Lesioning of PFX (0.3mA, DC current x 3 minutes) had no effect on seizures induced by SI stimulation (7/10 animals) using an avian-bolin immunocytochemical technique. Brain regions caudal to the inferior colliculus, such as the cerebellum and locus coeruleus showed no differences in the distribution of dopamine-β-hydroxylase-like immunoreactivity (DBH)- like immunoreactivity. However, differences in the distribution of DBH-I fibers were observed in more rostral brain regions including the central nucleus of the inferior colliculus, thalamus, hippocampus, piriform cortex, and somatosensory cortex. In these areas, the density, and intensity of DBH-I processes were lower in GEPR-9s as compared to SD rats. In other cortical regions no differences were observed. These results provide anatomical data that are similar to previously described biochemical results of the noradrenergic system in the brains of GEPR-9s. (Supported by NIH Grant NS-15659.)
EPILEPSY: SUBSTANTIA NIGRA AND AMYGDALA

THURSDAY PM

459.15


Analysis of anatomical structures underlying kindling is hampered by the time-consuming nature of this model. We therefore examined seizures induced by intra-amygdaloid microinjection of the convulsant, kainic acid. Behavior and EEG consequences were observed over 90 minutes. Kainic acid produced a dose-dependent (0.02-2 ug) increase in EEG and behavioral seizure intensity; the behavior mimicking that produced by electrical stimulation in kindled animals. Intra-peritoneal administration of the alpha-2 adrenergic antagonist, idazoxan (0.5mg/kg) markedly increased the seizure response to low dose (1 ug) kainic acid. Intra-nigral muscimol (50 ng/SN) attenuated the seizure response to high dose (2 ug) kainic acid. Microinjection of low dose (0.2 ug) kainic acid in animals previously kindled by intra-amygdaloid electrical stimulation evoked intense behavioral seizure mimicking high dose kainic acid.

Together these results indicate a marked similarity in the EEG and behavioral seizures evoked by chemical and electrical stimulation of the amygdala. Both seizures are controlled by substantia nigra and by an alpha-2 antagonist. Intra-amygdaloid injection of kainic acid may aid in elucidating the anatomical framework underlying kindling.

459.17

EFFECTS OF SMALL DOSES OF KETAMINE ON SEIZURE BEHAVIOR AND 2-DEOXYGLUCOSE UPTAKE INDUCED BY INTRA-AMYGDALOID STIMULATION. J.R. White*, K.M. Crenes and J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

As previously reported, low levels of electrical stimulation of the amygdala in awake, unrestrained rats produce graded seizure activity, as manifested by behavioral effects and 2-deoxyglucose (2DG) uptake (Crenes and Price. Neurosci. Abstr. 13: 1254, 1987). Low levels of stimulation, which did not produce convulsions, produced a pattern of 2DG uptake that reflected the majority of known primary projections of the amygdala. Higher levels of stimulation produced convulsive behaviors (e.g. wet-dog shakes), rearing and forelimb clonus as well as more widespread, often bilateral patterns of 2DG uptake.

Low levels of ketamine (1 to 10 mg/kg) were administered intravenously, in an attempt to reduce convulsive generalizations, while still using high levels of stimulation to evoke widespread 2DG uptake. The effect of ketamine was to reduce both convulsive behaviors and 2DG uptake, in a dose-dependent fashion. Saline control animals (1 to 3 mg/kg) moderated the expected pattern of 2DG uptake and reduced the intensity of the behavioral effects. Higher doses of ketamine (5 to 10 mg/kg) restricted the pattern of 2DG uptake to the heaviest primary amygdaloid targets, and prevented all but the mildest behavioral effects.

Supported by NHR AG05691 and NSH 59518. KMC is also supported by GM07950, Medical Sciences. LEW by Washington University Graduate Fellowship.

459.18


The effect of seizure activity on cognitive function was examined using the long-delay flavor-aversion paradigm in rats. Previous research has demonstrated a disruption of lithium-induced flavor-aversion conditioning with pentyleneetetrazol (PTZ) administered prior to lithium administration (Shaw and Webster, Psychopharm., 26:193-196, 1979). The present experiment was designed to determine whether this disruption was due associative (delay-independent) or mnemonic (delay-dependent) processes, and whether the reported disruption represents a functional consequence of all (i.e., chemically- vs electrically-induced) seizure activity. Saline treated rats receiving injections of lithium (0.9mEq) after consuming saccharin flavored water later avoided saccharin ingestion: the degree of avoidance varied inversely with the time (0.5 or 6 hr) separating initial saccharin availability and lithium injection. PTZ (50 mg/kg ip), administered just prior to lithium administration, impaired conditioning at both the long and short delays, suggesting a mnemonic deficit. Amygdala kindled seizures, elicited 2-3 min prior to lithium produced a disruption of conditioning at both the short and long delays, suggesting a more global, associative impairment. These results suggest a dissociation of the functional consequences of chemically and electrically elicited seizure activity.
TRAUMA II

460.1


Age is a major independent factor affecting head-injured patient outcome. Increasing age is associated with higher mortality and morbidity (J. Neurosurg., 68:1094, 1988).

We examined age effects on mortality and behavior after traumatic brain injury in rats. Three-month-old (young) and 20-month-old (aged) Fischer 344 rats were anesthetized with Metofane and injured at mild (1.75-1.85 atm) or moderate (2.0-2.3 atm) magnitudes of fluid percussion brain injury (J. Neurosurg., 67:110, 1987). Following injury, the acute duration of reflex suppression (e.g., pinna, corneal, righting, tail flexion) and long-term walking deficits (beam-walking) were recorded.

Mild injury produced mortality rates of 50% in aged and 25% in young rats. Moderate injury produced mortality rates of 100% in aged and 20% in young rats. Recovery of reflexes was faster for young rats. Aged rats exhibited greater and longer lasting motor deficits. Our findings suggest that fluid percussion injury to the rat may be a useful model for the experimental study of aging effects on brain injury.

Supported by NIH Grant 801-NS-21458.

460.3

TRAUMATIC BRAIN INJURY REDUCES QNB BINDING TO MUSCARINIC RECEPTORS IN RAT HIPPOCAMPUS. L. D. Oleniak*, B. G. Lyeth, T. J. Martin. Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Our laboratories have shown muscarinic agonist-receptor interactions may contribute to the pathophysiology of traumatic brain injury (TBI) (Brain Res., 466:1-13, 1988). One component of the pathology of TBI is the characterization of abnormal neuronal information flow which may be reflected by changes in receptor binding. The present study examined muscarinic receptor binding following TBI. Male Sprague-Dawley rats (280-340g) were either sham injured or given moderate fluid percussion brain injury (2.25-2.35 atm) (J. Neurosurg., 67:110, 1987). Animals were anesthetized with methoxyflurane and immersed in liquid N2 for 12-15 min. Brains were removed, cryoprotected in sucrose, and sectioned at 10-20 microns. Sections were incubated in 0.25% triton X-100-1% bovine serum albumin-0.2% formaldehyde-0.2% glutaraldehyde-100uM [3H]-QNB. Following a 2 hour incubation, sections were washed and autoradiographed with Kodak X-OMAT MR. Adult cats underwent intracerebral infusion of the extracellular marker, 14C-sucrose. Nine animals were given 2gms/kg of mannitol intravenously, and another 9 animals without mannitol were controls. Plasma and CSF osmolality were measured. After 2 hours the brains were removed for determination of water and electrolyte content and for preparation of the autoradiograms. Diffusion coefficients were calculated for intracerebral transport with equations for radial diffusion. Mannitol increased plasma osmolality and lowered water and potassium contents in the white matter. Diffusion was reduced in the direction of gray matter into the white matter. We conclude that low doses of mannitol control CSF pressure by selectively removing water from white matter, reducing CSF volume, and affecting molecular transport at the gray/white interface.

460.4


This study is an attempt to assess the effects on the nervous system of mice of different time-intensity combinations of microwave radiation. Current microwave exposure standards in the U.S. are not time dependent, whereas many other countries do incorporate a time factor into their standards.

Carbon loaded teflon cortical screws were implanted in HS mice one week prior to exposure. Experimental mice were placed in a restraint located at an H field maximum of an S band waveguide. This precision of placement within the field allowed us to determine mid-brain SAR's accurately. "Yoked" control mice were similarly restrained and placed in a sham waveguide. The microwave signal was 2450 MHz pulsed and the specific absorption rates(SAR's) used were 1.0 and 10 mW/g. Spontaneous electrocorticograms and EEG's were taken. Changes in EEG's were noted as a decrease in frequency and an increase in the amplitude of the evoked responses. In both indices, a short term (<30min) large, time-intensity reciprocity was recorded as well as a smaller, long term reciprocity. The results indicate that exposure standards need to be based on a reciprocal relation between time and intensity of microwave radiation. Further detailed studies should specify the nature of such a standard.

460.5

NEURAL NETWORK COMBINATORICS: IMPLICATION FOR RECOVERY FROM BRAIN DAMAGE. R. B. Glassman, Dept. of Psychology, Lake Forest College, Lake Forest, IL 60445.

The factors determining whether a neuron in a particular set is active include its threshold and how many connections converge onto it from neurons in another set. Consider a simplified general case of a hypothetical set of N neurons, which constitute a source of input. Each possible combination of M of these N neurons is connected to a type operation. The formulas also provide a way to estimate reliability and to test the sacrifice of discrimination associated with arousal or with threshold adjustments compensating for the shock or "dissection" component of a defect that follows brain damage.

460.6


Carbon loaded teflon cortical screws were implanted in HS mice one week prior to exposure. Experimental mice were placed in a restraint located at an H field maximum of an S band waveguide. This precision of placement within the field allowed us to determine mid-brain SAR's accurately. "Yoked" control mice were similarly restrained and placed in a sham waveguide. The microwave signal was 2450 MHz pulsed and the specific absorption rates(SAR's) used were 1.0 and 10 mW/g. Spontaneous electrocorticograms (EEG's) were taken. Changes in EEG's were noted as a decrease in frequency and an increase in the amplitude of the evoked responses. In both indices, a short term (<30min) large, time-intensity reciprocity was recorded as well as a smaller, long term reciprocity. The results indicate that exposure standards need to be based on a reciprocal relation between time and intensity of microwave radiation. Further detailed studies should specify the nature of such a standard.
460.7 


A single administration of d-amphetamine (AMPH) to rats was given 24 hr after sensorimotor cortex ablation (see SCI Critical Reviews in Neurobiol., 2:253-260, 1980) combined with relevant experience during the period of intoxication produces an enduring acceleration of recovery of locomotion. This study tested methylenphenidate (MDP) in a rat model of hemiplegia.

After training rats to run a narrow elevated beam, a right-sided sensorimotor cortex ablation operation was performed. Twenty-four hr after surgery, 3, 6, 9, 10, 12, or 15 mg/kg of MDP or vehicle was given (i.p.). Beam-walk testing resumed at 1, 2, and 6 hr post-injection and every other day for 15 days. 

No significant effects of a single dose of MDP was obtained compared to saline controls. The reported beneficial effects of MDP on cognitive function after brain injury (J. Clin. Exp. Neuropsychol., 2(2):297-309, 1987) may require continuous medication. Additionally, this rat hemiplegia model is inadequate for screening drugs affecting cognitive deficits. Supported by NIMH Grant NS02220-02 and by NERS 3006 R090129-14.

460.9 


Traumatic brain injury (TBI) is a major cause of disability and mortality in the United States. The cognitive, emotional, and social changes that result from TBI can be severe and long-lasting, affecting both the individual and their family and community. This study investigated the impact of rehabilitation on cognitive, social, and emotional performance within each group and between acute and chronic TBI's. Issues of critical time for rehabilitation intervention, treatment efficiency, capacity for integration and reorganization are raised.

461.11 


We have previously suggested that endogenous κ-opioid receptor systems may be involved in the pathophysiology of traumatic brain injury (E. H. You, T. D. Van, J. R. Two-Olivares, M. J. Chen, and D. W. Neuman. Depts. of Psychol. and Physiol., UNM, Albuquerque, New Mexico 87131). 

We compared the efficacy of vascularized (VASC) and conventional (CONV) sciatic nerve grafts in restoring nerve conduction, the blood-nerve barrier, noradrenaline (NE) and 6-keto prostaglandin F1α (6-KPGF, the stable prostacyclin metabolite) in the rat. We also measured malondialdehyde (MDA) content. There was a statistically non-significant increase in nerve action potential amplitude in the vascularized segments of vascularized nerves at 1 and 2 months post-graft. The NE 6-keto-aracenic PA product was increased in both VASC and CONV at 1 and 3 mo, but not different to each other. NE and 6-KPGF, the major vasoconstrictor and dilator of nerve microvessels were better restored in VASC CONV. A statistical significance for each group (p<0.001). MDA used as a marker of oxygen free radical generation was not significantly different in the 3 groups. Group NE MDA (ng/mg) PA (pg/mg) (pg/mg prot) dry wt (10^-9/sec). 

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (ng/mg)</th>
<th>PA (pg/mg)</th>
<th>(pg/mg prot)</th>
<th>dry wt</th>
<th>1 mo</th>
<th>3 mo</th>
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<tbody>
<tr>
<td>CTL</td>
<td>2.6±0.18</td>
<td>8.3±0.59</td>
<td>5.8±0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VASC</td>
<td>2.1±0.26</td>
<td>6.7±0.49</td>
<td>6.6±1.02</td>
<td>11.6±1.5</td>
<td>11±1.9</td>
<td>1.6±0.7</td>
</tr>
<tr>
<td>CONV</td>
<td>1.9±0.37</td>
<td>4.0±0.32</td>
<td>6.7±0.77</td>
<td>9.6±2.2</td>
<td>9±1.7</td>
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</tr>
</tbody>
</table>

The better restoration of 6-KPGF and perhaps NE suggest that vascularized grafts may be more effective in restoring vasoactivity of peripheral nerve following graft.

460.8 


Twenty-four male Sprague-Dawley rats approximately ninety days of age received either bilateral medial-frontal cortical lesions or sham operations. Subjects received liposomes (SRI International) with or without physiologic amounts of α-tocopherol (αT) (Kodak). Following the injury, 0.1 cc of the treatment material was flushed directly into the wound cavity. The treatment was delivered directly to the lesion site by PE60 tubing attached to subcutaneously implanted Alza Alzet pumps (Model 2001). After seven days of continuous treatment, the pumps were removed and subjects were treated for seven days. Subsequently, rats were trained on a delayed-spatial alternation test for a water reward. The results of this study showed that, in comparison to sham operations, brain-injured subjects given liposomes alone were significantly impaired on this spatial task. However, rats with lesions treated with αT-containing liposomes took significantly fewer days and made fewer errors than lesion controls in attaining 9 out of 10 correct responses. Histological analyses will be described. This research is supported by NIMH Grant No. ROI NS258658.

460.10 


This model of traumatic hemiplegia is inadequate for screening drugs affecting cognitive deficits. Supported by VA Merit Review 74R.
PRETREATMENT WITH A-4 REDUCES BEHAVIORAL DEFICITS FOLLOWING IMPACT SPINAL CORD TRAUMA IN RABBITS.

M. Lenke, F. Demediuk, and A.I. Faden (SPON: T.F. Jacobs). Dept. of Neurology, University of California, V.A. Medical Center, San Francisco, CA 94122.

Secondary neurological events following traumatic spinal cord injury (SCI) appear to contribute to development of the injury severity. Membrane lipid hydrolysis and eosinophilic production occur after SCI in cats and rats. In the present studies changes in water content (wet/dry weight), tissue Na+, K+, and water of phosphorylation and receptor activity, Mg2+ changes after trauma may play a fundamental role in secondary tissue damage.

REGIONAL CHANGES IN GLUCOSE AND ATP CONTENTS FOLLOWING EXPERIMENTAL SPINAL CORD COMPRESSION TRAUMA IN THE RAT.

A.C. Nacimiento, G. Adler*, and A. Mautes* Neurosurgical Research Laboratory, Searle University Medical School, 6650 Humbert/Seate, F.R.G.

To study regional metabolic aspects of acute posttraumatic changes in the spinal cord following compression injury, we used the model of Nacimiento et al. (Neurosurg., 1985, 62, 899) we applied a bioluminescence technique described for brain tissue by Pace-Asciak et al. (J. Cereb. Bl. Flow Metab., 1985, 5, 465). Correlation between computer-assisted measurements of optical densities in bioluminescent images of glucose and ATP distribution in the spinal cord sections, and contents in corresponding tissue samples was highly significant. Thus, both metabolites could be quantitatively determined by image processing of the bioluminescent sections. Two hours after compressing 60 % of dorsoventral extent of segment L4 for 50 ms, we found significant elevation in glucose and reduction of ATP content in L4 and L5 segments. Changes were regionally significant. This method disclosed regional differences in posttraumatic carbohydrate and energy metabolism which cannot be resolved by measurements in tissue samples.

EXPERIMENTAL OBSTRUCTIVE HYDROCEPHALUS: SEQUENTIAL MRI'S OF RAT BRAINS OVER A 10-DAY TIME COURSE SUGGEST AN AGE-RELATED DIFFERENTIAL RESPONSE IN ABILITY TO RESOLVE OBSTRUCTIVE HYDROCEPHALUS.

Kathryn A. Mager, Juan A. Cabrera, W.B. White and Michael E. Miner, M.D., Ph.D., Division of Neurosurgery, University of Texas Medical School, Houston, TX, 77030.

In producing a reliable model of hydrocephalus in rats, the question of variation of age of the animal and effect this may have on the course of hydrocephalus has not been adequately addressed. It is the purpose of this study to begin to contrast these responses. Obstructive hydrocephalus was produced in 45 and 64 day old male, Sprague Dawley rats weighing approximately 150 g, and 250 g, respectively, by injection of kaolin into the cisterna magna. Each animal brain was imaged via the utilization of MRI at 2,4,7, and 10 days post-operatively to follow the progression of ventricular dilation and subsequent neurological deficit. Our findings indicate that the age-related differential response represents an important variable affecting the outcome in experimental obstructive hydrocephalus studies.

We have previously examined the development of acute pathological changes after contusive injury in the rat spinal cord. These pathological changes are mainly characterized by changes in the volume and volume ratio of normal axoplasm that is similar in mild and severe injuries. This is further reduced between 15 mins and 2 hrs after which it remains virtually constant through 8 weeks after contusion. Thus, in both mild and severe injury the significant axonal pathological alterations that lead to axonal loss appear to have been accomplished by 2 hrs after trauma. In addition, examination of the chronic injury sites at 8 weeks also indicate a preferential loss of the larger myelinated axons and increased myelin thickness for the remaining small axons as compared to unlesioned spinal cord. (Supported by NIH-NO 1-NS-2-2310)


Using microdialysis, changes in extracellular concentration of amino acids and inorganic ions ([K+]e and [Ca2+]e) following fluid-percussion brain injury were determined in the rat hippocampus. At the moment of injury, the microdialysates contained a high concentration of excitatory amino acids, especially glutamate, which was noted (4-5 fold increase and the [Ca2+]e decrease were resistant to in situ administration of tetrodotoxin (10-4 M) through the microdialysis probe but effectively attenuated by kynurenic acid (10-4M), a glutamate antagonist. These results suggest that a release of excitatory amino acids underlies the massive ionic fluxes following concussive brain injury.

EXOGENOUS LAMININ/ARA C PROMOTES "REPAIR" OF SPINAL CORD. Y. Miller and M. Pollia (C.Fischette spon.), Ortho-Surg., U.B.C., Vancouver V5Z 4H4

Exogenous laminin can support axon elongation in vivo. Administration of Ara C (cytosine arabinofuranoside) after CNS injury inhibits reactive gliosis. In the present study, these approaches were combined after rat spinal cord injury. Cord were subjected to contusion lesion at T8. Elvax pellets containing laminin were inserted subdurally over dorsal columns and Ara C administered by daily i.p. injection. Rats were assessed behaviorally and morphologically for 214po. Behavioral studies (including plane test and assessment of gait and toe spread) indicative significant 'recovery' when laminin pellets were inserted after spinal cord transection. However, lower doses of laminin were used. Results were corroborated by elongating axons in dorsal columns adjacent to lesion site in vivo. Electrophysiological studies are in progress.

Studies are underway to determine if laminin/Ara C treatment can be used in combination with other interventions. Studies to date indicate no synergistic effects when combined with exogenous D.C. current.

TWO NEW PHARMACOLOGIC AGENTS WHICH PROTECT THE RAT SPINAL CORD FROM COMPRESSION INJURY. B. Thorton Ohnishi, Hironori Katagi* and Masayuki Katayaoku*. Membrane Research Institute, Philadelphia, PA 19104.

Using the weight-drop technique (10g x 5cm), we studied the protective effect of two new drugs in spinal cord injury of the rat. The efficacy was assessed by recovery of motor performance (Tarlov score) during 4 week period after injury.

(1) Chrysothromacin, a specific inhibitor of Ca-activated K efflux, demonstrated protective effect in the present study (0.12 mg/kg i.p.; 30 minutes before injury). Quinidine also had a beneficial effect.

(2) Pharmacokinetics of rosiglitazone (5 mg/kg of either MR-256 or MR-356; i.p., post-injury administration), which may act as inhibitors for phospholipases and proteases, also had protective effect. Possible mechanisms of protection are speculated.
461.1

NTX increased LC discharge 55 ± 11% (n = 6) at 30 min after NTX injection, compared to saline control animals. NTX also reduced 30 min post-injection arterial blood pressure and opiate-induced muscle rigidity. These results show that NTX acts on intact brain tissue to precipitate opiate withdrawal.

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Previously, we have demonstrated two effects of morphine on adenylate cyclase activity (AC). NTX inhibited AC activity and morphine increased AC activity. In this study, we have identified a novel decrease in AC activity evoked by repeated sciatic nerve stimulation specific to the LC. This may be a general feature of opiate withdrawal, indicating a role for increased AC activity in the development of opiate tolerance and dependence.

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Although specific sigma binding sites are present in brain, studies on the effects of sigma-directed ligands on neurons have been lacking. Largest et al. (Purif. Pharmacol., 27, 1986) showed that hybrid cells (mouse neuroblastoma - Chinese golden hamster brain) of the NCB-20 line show high affinity sigma binding with a Kd of 1.0 µM. We have obtained intracellular recordings from these cells with micropipettes (30 Mohm) and studied the effects of pressure ejection of drugs from the pipette. We have observed an increase in spontaneous discharge rate that was blocked by haloperidol.

461.4
MPV 1440, A HIGHLY SELECTIVE ALPHA-2 AGONIST, PREVENTS OPIATE-INDUCED MUSCLE RIGIDITY. M.D. Haniger, I.S. Segal*, and M.M. Maze*. Deps. of Anesthesiology, Univ. of California, San Diego, Stanford University, and the San Diego and Palo Alto V. A. Medical Centers. Several studies have shown that the selective alpha-2 adrenergic agonist, MPV 1440, has marked anagelisa, muscle flaccidty and decreased sympathetic activity.

Previously, we have demonstrated two effects of morphine on adenylate cyclase activity (AC). NTX inhibited AC activity and morphine increased AC activity. In this study, we have identified a novel decrease in AC activity evoked by repeated sciatic nerve stimulation specific to the LC. This may be a general feature of opiate withdrawal, indicating a role for increased AC activity in the development of opiate tolerance and dependence.
461.5 OPIATE-MEDIATED BEHAVIOR AND BRAIN OPIOD PEPTIDES IN MONONGELES AFTER PREGNATAL ETOPONIC, P. H. Venclovas-Jakuba and V.J. Shenoy. Trinity College, Hartford, CT & U. Conn. Health Center, Farmington, CT.

Pregnant rats were fed either an alcohol-containing diet, an isoaloric pair-fed diet or standard chow. At birth all offspring were fed food to control mothers eight per litter, and tested at ten days of age. Similar groups were sacrificed at 12 days of age for brain B-endorphin levels using a specific radioimmunoassay. Ten-day-old rats were isolated and monitored for ultrasonic vocalizations after naltrexone (0.5mg/kg) administration. After 10 min isolation they were tested for analgesia to heat. Both-pair fed and pair-fed saline controls vocalized 200 times during isolation, compared to 35-50% of controls double with naltrexone pretreatment. Alcohol-treated pups, however, vocalized significantly less after saline (75) and naltrexone (150). Additionally, these pups did not demonstrate the isolation-induced analgesia seen in controls but did show a marked increased sensitivity to heat after naltraxone administration. Prior studies have demonstrated that prenatal ethanol results in markedly increased levels of B-endorphin at birth. Physical and psychological effects may be due to a primary defect in B-endorphin levels or, secondarily, to abnormalities of the opiate receptors.

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Agonists for three opioid receptor subtypes, δ (leucine-enkephalin) and μ (morphine) were studied in 3 to 7 fetal sheep 100 to 124 days gestation. Naloxone, L.Ahtee and K. R. Carlson. Dept. of Pharmacology, Wayne State Univ., Detroit, MI 48202.

Morphine had no effect on mean arterial pressure (MAP) or heart rate (HR) in doses up to 10 mg/kg. Leucine-enkephalin (LEK) produced a brief, significant decrease in MAP and HR, which was abolished by vagotomy, similar to effects previously reported for met-enkephalin (LaGamma et al., Pediatr Res 17:162-67, 1983). Dynorphin-13 (DYN) produced a significant increase in MAP which lasted up to 85 min, and was not abolished by vagotomy. DYN had no effect in HR in intact fetuses, but produced a significant increase in HR in vagotomized animals. These results suggest differential control of CV function by at least two endogenous opioid systems in developing sheep. Research Supported by NIH Grant HL34460 to CED.

461.7 DISINHIBITION OF PITUITARY LH BY INTRACEREBRAL ANTI-OPIOID IMMUNOGLOBULINS IN SHEEP. G.D. Weissman* and P.Y. Malve, Dept. Animal Sci., Purdue Univ., W. Lafayette, IN 47907.

This research sought to determine if β-endorphin (BE) exerts a physiological effect on LH release in sheep. Previous studies have not been evaluated to date. In this report we present data concerned with the effects of morphine on steady-state release in the CNS of sheep. Nine mature ewes were implanted with guide tubes through which matched infusion cannulae could subsequently be briefly inserted for intracerebral infusions of saline (9.3 μl in 20 μl), normal sheep serum (NSS, 20 μl of 1:25), or sheep anti-sheep βE antisera (SAE; 20 μl of 1:25). To detect abrupt diminution of LH release after opioid treatments, serum LH was quantified at 10-min intervals for 1.5 h before and after each infusion. During the luteal phases of recurring estrous cycles, complete experiments i.e., 3 different infusions at each site over 27 h period were performed 2-4 times/site. HH assays and histological analyses of CNS sites showed that SAE consistently diminished LH/LHRH release when infused at the following sites: (A) rostral preoptic area/nucleus accumbens (2 sites) and (B) anterolateral hypothalamus (1 site). Infusion of NAL also diminished LH/LHRH release at site A but not at site B. In addition, NAL inhibited LH/LHRH release at 5 other sites (3 in areas slightly rostral to site A and 2 in medio basal hypothalamus), but serum LH responses to SAE were not consistently different from those to NSS. Three other sites were not responsive to either NAL or SAE. In summary, immunoneutralization of endogenous BE in selected CNS sites relieved the inhibition of LH release. However, other opioid ligands may also be physiologically important in luteal-phase sheep since the non-specific anti-opioid NAL was effective at more sites than SAE.


The excitatory effects of opioid agonists on SLA thus appear to originate from an activation of mu receptors. The excitatory response is of an established central origin and elicited by drug administration of fenfluramine.

461.9 AUGMENTATION OF MORPHINE-INDUCED CHANGES IN CEREBRAL MONOMERIC METABOLISM IN RATS TREATED CHRONICALLY WITH NALTREXONE. L.Abele* and R.L. Carlin*, Dept. of Pharmacology, Univ. of Helsinki, SF-00170 Finland.

Chronic naltrexone treatment enhances the antiinhibitory effect of morphine as well as the binding of opioid ligands to δ-sites (Temptel, A. et al., J. Pharmac. exp. Ther. 232: 439, 1985). To investigate the role of opioid receptors in the regulation of cerebral monomeric neuropeptide we studied the effects of morphine (3-30 mg/kg, 2 h, s.c.) on the concentration of DA, NA, 5-HT and their metabolites in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps) and decreased their metabolism in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps) and their metabolites in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps) and their metabolites in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps) and their metabolites in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps) and their metabolites.

Chronic naltrexone treatment elevated the concentration of 5HT in the hippocampus, the hippocampus and the lower brain stem, that of NA in the lower brain stem and decreased the concentration of 5HT in the striatum. In naltrexone-treated rats morphine elevated the cerebral dopac, HVA, MEHP and 5HTA concentrations as well as concentrations of the striatum in the cortex, pyriform cortex and N accumbens. In contrast, DA release in the striatum was unchanged after treatment with morphine. These data support previous observations that morphine acts to uncouple DA synthesis and release in the rat striatum but that this uncoupling is not present in other DA projections to cortical and limbic terminal fields.

461.10 EVIDENCE FOR MU RECEPTOR AND 5-HT LINK IN EXPRESSION OF MORPHINE INDUCED HYPERACTIVITY. S. Gotz. Department of Pharmacology, K.G. Medical College, Lucknow, 226003, INDIA.

Opioid agonist morphine, in high doses, induces hyperactivity in rodents and other species. Intraperitoneal (ip) administration of morphine (30mg/kg) consistently evokes marked enhancement of spontaneous locomotor activity (SLA) in naive mice. This naloxone antagonizable, excitatory response is of an established central origin but the opioid receptor subtype involved in this action is yet to be distinctly recognized. We tested opioid agonists and agonist-antagonists for SLA potentiating effects in naive mice, using photoacometer. Pentazocine, a mu receptor antagonist and kappa and sigma agonist, did not evoke any increase in SLA after administration of less than 60 mg/kg, ip, while a relatively selective mu agonist, fentanyl, (0.5 mg/kg, ip) produced marked hypermotility. Prior administration of pentazocine (30 mg/kg, ip) blocked morphine as well as fentanyl induced potentiation of SLA. The excitatory effects of opioid agonists on SLA thus appear to originate from an activation of mu receptors. Additionally, the manifestation of these mu receptor mediated effects requires an intact serotonergic system since both morphine and fentanyl failed to augment SLA in mice in which serotonin had been depleted by prior administration of fenfluramine.
ALTERATIONS IN POSTSYNAPTIC B-ADRENERGIC FUNCTION

We used single unit recording techniques to examine the effects of striatopallidal enkephalin and GABA containing neurons. 20% of GP cells studied (n=20) were inhibited >80% baseline, 40% of cells >50%, 10% of cells >20%, and 55% of cells were not significantly affected. Similarly, the sensitivity of GP cells to CABA was significantly decreased in lesioned rats. In noloxone treated lesioned rats, the reduced sensitivity of GP cells to DADLE was completely reversed whereas the sensitivity of GP cells remained subselective to GABA. These data show that 6-OHDA lesion causes a functional down regulation of opioid and GABA receptors in GP and that the former change is reversed by chronic noloxone treatment. The data suggest that dopamine denervation causes increased activity of striatopallidal enkephalin and GABA containing neurons.

EFFECTS OF MONOAMINE ANTAGONISTS ON OPIOID-INHIBITION OF MILK-EXJECTION REFLEX IN THE RAT

We recently reported that β-adrenergic receptors are increased in various brain areas in rats after chronic morphine treatment and subsequently dose-dependent during opioid withdrawal (Brain Res. 400, 1987). In this study, measurements of [3H]-dihydroalprenolol (DHA) binding in hypothalamic and cerebral cortex structures were carried out together with measurements of [3H]-MAA binding in conjunction with electrophysiological recording in vivo to determine whether such adjustments in receptor density might be of functional significance. Alterations in postsynaptic β-adrenergic receptor function were assessed by comparing the ability of isoprenaline (IS) to increase population spike responses and to block the calcium-dependent potassium after-hyperpolarization (AHP) in pyramidal neurons of the hippocampus and cerebral cortex (Lieberman and McLennan). Treatment of rats with increasing doses of morphine (up to 100μg/kg), t.i.d.) for 14 days resulted in a 19% in the Ilex (for [3H]-DHA), whereas B-receptor density was decreased to 85% in withdrawn subjects. No changes were found in the affinity of the receptors for [3H]-DHA or the agonists NE and ISO. Electrophysiological recording in CA1 revealed that extracellular responses to threshold (25mV) as well as maximal concentrations (500mM) of ISO were significantly enhanced in slices from dependent rats, whereas responsiveness to maximal concentrations of ISO was decreased, relative to controls, in slices from withdrawn subjects. In addition, the EC50 for ISO in reducing the AHP was significantly lower in lesioned withdrawn groups compared with non-lesioned slices (2.2μM), respectively, than for controls (200μM). These data suggest that long-acting opioid administration and opioid withdrawal are accompanied by functional alterations in postsynaptic β-adrenergic systems (supported by NIDA grant DA-03005).
461.7 OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS IV

NAALADase inhibitors on [$3H$]NAAG metabolism in vivo, consistent with a role in endogenous NAAG disposition.

461.8 MODIFICATION OF PERIPARTURITIONAL PAIN THRESHOLD BY INGESTION OF AMNIOTIC FLUID IN THE RAT. B.L. Stauch, M.B. Robinson, G. Forloni and J.C. Doerr*

Results: (a) larger doses produce greater enhancement; (b) the longer the interval between the prepartum test and the onset of delivery, the greater the enhancement. Prepartum AP lowers pain thresholds during parturition and after delivery, if the dose of AP is low and the IPDOD is short. Naloxone pretreatment lowers pain thresholds during (a) the prepartum, parturitional, and postpartum period in control rats, and (c) the parturitional and postpartum period in control rats with short IPDODs.

Mother rats show increased analgesia resulting from ingestion of amniotic fluid in the periparturitional period. The enhancement is complex; however, POE may have a biphasic action, and may even enhance opioid antagonists such as naloxone.

462.1 EFFECTS OF NAALADASE INHIBITORS ON [$3H$]NAAG METABOLISM IN VIVO. E.L. Stauch, M.B. Robinson, C. Forloni and J.C. Doerr.

In the rat, gonadal steroids influence central and peripheral levels of the endogenous opioid peptide, beta-endorphin. As an assessment of total endogenous opioid activity, the time to respond to a noxious somatic stimulus (tail flick latency and paw lick/jump latency) was measured in unanesthetized and castrated gonadal steroid replaced male and female rats. Rats were castrated for two weeks prior to testing. At the end of the third week, gonadal steroid treatment was initiated and maintained for three weeks in half of the animals, while the remaining animals received the vehicle and served as castrated controls. The tail flick latency was significantly increased in castrated male rats at 1, 2, and 3 weeks of treatment with TP. In castrated female rats the tail flick latency was increased 2 and 3 weeks after estradiol benzoate (EB) treatment was begun, while the time to paw lick or jump was reduced with 2 and 3 weeks of treatment with EB. These data indicate that gonadal steroids modulate the perception of pain in male and female rats and that this modulation may vary according to sex and the stimulus employed.
PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION IV

Acetic acid extracts of human brain tissues or CSF from FGIN, George town Univ., Washington, D.C. 20007. rabbits against fragment 51-70 (eikosaneuropeptide, ENP), rat brain DBI is processed into 3 biologically active fragments, one of which is identical to plasma L-major acute protein (L-MAp). The latter increases in adjacent rat brain treated with intravenous administration of HCNAAG followed by one hour with Krebs buffer devoid of Ca2+, containing 1mM EGTA. The basal level after labeling with HCNAAG appears to be associated predominantly with glutamate, and verandine depolarization has little effect on this release. Prior encision of the right eye reduced the basal level of [3H]NAAG release by 50%, and the evoked evoked release by greater than 85%, from the left superior colliculus. Preliminary studies indicate that [3H]glutamate subjected to the same procedure of labeling, washout and evoked release results in less than two-fold increase in release of tritium associated with NAAG on HPLC. Thus, with [3H]NAAG as the substrate, a more specific labeling of a discrete population of [3H]NAAG, which was virtually abolished by co-perfusion with Krebs buffer containing 1mM Ca2+ and 1mM EGTA, was used as a precursor. These monoclonal antibodies stained numerous nuclei of the medulla-pons and midbrain, mitral cells in the olfactory bulb, and pyramidal neurons in sensorimotor cortex, locus coeruleus and several cholinergic cranial nuclei. The staining pattern strongly correlated with NAAG levels determined by HPLC. Monoclonal antibodies significantly enhanced sensitivity of staining, allowing visualization of dorsal horn neurons in spinal cord which were not readily detectable with polyclonal antisera.

Availability of these monoclonal antibodies now facilitates further clarification of the role of NAAG in the brain.

Calcium-dependent evoked release of [3H]N-acetyl-D-aspartyl glutamate (NAAG) in rat brain is a neurotransmitter/neuromodulator in the optic tract.

To interpret in structural terms interactions of rat DB, with BZ recognition elements, we used secondary structure prediction methodology (Chou, P.Y., Fasman G.D., Biochemistry 13: 222, 1974) and analysis of hydrophobicity (Kyte, J., Doolittle, R.F., J. Mol. Biol. 157; 105, 1982). We synthesized the DBI peptides (17-50), (18-50), (22-50), (26-50) and (33-50) by solid phase methodology. GC spectrum of DBI (17-50), (18-50), (22-50) demonstrated a helical structure in MeOH. Pharmacological studies revealed that these peptides injected intraventricularly in rats cause convulsant activity. In primary cultures of cerebellar granule cells DBI peptide (33-50), displaced [3H]flumazenil with K_i of ~ 5 uM, the other synthetic peptides were ineffective in concentrations up to 200 uM. In primary culture of the cerebellar granule cells the peptides with stable helical structure DBI (37-50), (38-50), (22-50) were able to displace [3H]KI11195 with K_i ~ 10 uM. DBI (26-50) and (33-50) which lack the helical part of their structure were ineffective.


L-aspartate N-acyltransferase (ANAT), a nervous system specific enzyme, mediates the acetylation of L-aspartate by acetyl-coenzyme A. The product, acetyl-L-aspartate, is a predominant acidic amino acid in the nervous system and may serve as a precursor in the biosynthesis of acetylcholine and other neurotransmitters.

ANAT was solubilized from brain membranes in 1% Triton X-100 in 10mM phosphate buffer, pH 6.8. The solubilized enzyme passed through a DEAE-anion exchange column at pH 7.0 and the unbound fraction, containing most of the enzyme, was further purified by anion exchange HPLC at pH 8.1. The active peak fractions were pooled and subjected to gel filtration HPLC followed by affinity chromatography on an L-aspartate immobilized column. The eluate showed a 500-fold increase in specific activity and 2-3% yield.

This purification will support analyses of the regulation and cellular distribution of ANAT, contributing to an understanding of the function of N-aspartylaspartate. (grant DA 02597)

462.12 NEWLY SYNTHESIZED PEPTIDE IS FIRST TRANSPORTED TO RELEASE SITES: AUTORADIOGRAPHIC EVIDENCE FROM A CRAB NEUROSECRETORY SYSTEM. E. Stuenkel, E. Gillary*, and I. Cooke. Békésy Laboratory of Neurobiology, and Dept. of Zoology, University of Hawaii, Honolulu, HI 96822.

Preferential release of newly synthesized peptide has been found for all neurosecretory systems tested. This study shows that granules containing biosynthetically radioactively labeled material are first transported to release sites. Later they become intermixed with non-labeled granules. The X-organ - sinus gland system of crabs (Cardisoma crinipes) was isolated and arranged to permit independent perfusion of the somata (X-organ) and terminals (sinus gland). The somata were given a pulse (5 min to 3 h) of [3H]-leucine in crab saline with glucose, while the terminals were continuously perfused with nutrient medium. Systems were chased for 1–72 h. The material was examined by light- and EM autoradiography (7 exposures).

At 1 h, exposed grains occur primarily over granules in Golgi. At 10 h, radiolabel is in the axon tract with little in the terminals. By 10 h, label is found preferentially in terminals abutting the internal hemolymph sinuses. After 72 h, label is evenly distributed throughout release and storage sites.

Exocytotic profiles, indicative of release sites, have only been observed where terminals abut a hemolymph sinus (Weatherby, 1981, Cell Tissue Res. 220:293). Transported label represents hormonal peptides, and these are secreted by a Ca-dependent mechanism (Stuenkel, 1983, J. Comp. Physiol. B153:191; 1985, J. Physiol. 359:163).

Thus, the autoradiography becomes a physical basis for preferential release of newly synthesized peptides. Supported by NSF BNS84-04459 and NIH NS 15453.


Radioimmunoassays for neuropeptides and cyclic-AMP are time consuming because they are based on radiolabeled compounds with segregation of a mixture performed by antibodies. Liquid chromatography can resolve a mixture of neurotransmitters but not for some applications. We overcame some of these difficulties using high performance capillary electrophoresis (HPCE). Separation of a mixture of peptides and cyclic-AMP was performed in an unsilicized fused silica capillary tubing (75 µm x 100 cm) using a mobile phase of 0.5 M sodium tetaborate, pH 8.3. The test mixture contained neurotransmitters, met-enkephalin, leu-enkephalin, substance P, angiotensin II, sulfated cholecystokinin and cyclic-AMP. Detection was performed with a modified Hitachi UV detector. Samples (4 µl) were electrophoretically loaded applying 10KV for 15 sec, and electropherograms were run for 10 minutes at 20 KV. By measuring polarities the peptide zones were run forward and backward to determine the UV-spectrum of each substance. Linearity was tested with eight different concentrations of the mixture (range 10^-4 M to 10^-6 M). For all compounds the regression coefficient relating concentration of the standard to absorbance units was equal to 1.00. Compounds were well separated and the retention time error was less than 2% in nine different runs. Sensitivity was between 0.1 and 0.01 picomoles, and quantification of a sample lasted about 20 min per run. These results show that HPCE is reliable for neuropeptide and cyclic-AMP analysis.


Distribution of rat AG mRNA and in particular, the cell types associated with AG mRNA in brain, was determined by using in situ hybridization combined with immunocytochemistry in single brain sections. Two hybridization protocols were used: a 35S-labeled 42 residue oligonucleotide and a full-length AG RNA (sense or antisense) labeled with tritiated uridine. Rats were perfused with paraformaldehyde (10% w/v), pH 7.4 and perfused transcardially with 0.9% sodium chloride, 0.9% glucose and 0.05% sodium azide. Brains were removed, cryoprotected and sections cut 10 µm thick on a cryostat and placed into prehybridization solution for 4-18 hours. A 35S-labeled oligonucleotide or full-length sense or antisense AG mRNA was added to the solution at a concentration of 3.3 μg. Thirty-50 hour exposure onto x-ray film labeled 42-mer oligonucleotide was added to some sections as a control in the first experiments. Sections were then treated with 10µg/ml of RNase A and 0.2 µl/ml of RNase T1 for 2 hours at 37°C, washed with 0.05 M Tris-HCl, pH 7.4, and hybridized overnight at 37°C followed by a 42°C wash (both washes). Sections were then dipped in NTB2 emulsion and exposed at 4°C, washed with 0.05 M Tris-HCl, pH 7.4, and developed with D-19. Sections of silver grains were seen throughout the brain with higher concentrations in ION, NTBM, and many hypothalamic nuclei. The highest concentrations are mainly clustered over GFAP reactive cell soma and processes. Most gran cell clusters were not associated with MAP reactive cells. This confirms our previous findings that the distribution of AG mRNA overlaps many of the areas reported to contain high levels of angiotensinogen and further suggests that angiotensinogen in brain is synthesized primarily by glia.
462.1


Alpha bag cell peptide (α-BCP), one of the multiple secretory products of the precursor to the eeg-laying hormone (α-ELH) of bag cells, has been reported to exert excitatory (Rothman, et al., PNAS, 80:3753, 1983) or inhibitory (Kauer, et al., J. Neurosci., 7:93, 1987) feedback on the cells which secrete it. Since pro-ELH synthesis in these cells is modulated by cAMP, it was of interest to determine if α-BCP causes parallel alterations in cAMP levels and precursor synthesis. In this study, the peptide had no effect on unstimulated bag cell cAMP levels or on elevation of cAMP levels by TMG, indicating a lack of effect on basal adenylate cyclase activity. However, it reduced the cAMP elevations induced by forskolin, high potassium, and dopamine. Consistent with the hypothesis that pro-ELH synthesis is accelerated by cAMP, α-BCP reduced precursor synthesis and cAMP production in parallel in forskolin-stimulated preparations, but did not reverse the stimulation of peptide synthesis caused by TMG or depletion of intracellular Ca**.

MESSENGER RNA REGULATION IV

463.1

C-FOS mRNA EXPRESSION IN BRAIN INDUCED BY SUBCONVULSIVE DOSES OF SEIZURE EXCITATION. T. Nakajima, J.-L. Daval, B.P. Morgan, and P.J. Marangos. Unit on Neurochemistry, NIH, Bethesda, MD 20892 and 1Genesia Pharmaceuticals, San Diego, CA 92121-1207.

In several previous reports, the proto-oncogene c-fos was shown to be induced by occupation of nicotinic acetylcholine receptors and depolarization-induced calcium influx in PC12 cells. It is also induced following various seizures. Caffeine is a non-selective adenosine receptor antagonist as well as a convulant at high doses. We examined caffeine-induced c-fos expression and its pharmacological interaction at the mRNA level in mouse brain. Caffeine administration (17.5mg, 32mg, 56mg, and 100mg/kg i.p.) increased dose-dependently c-fos mRNA levels and at the convulsive dose (175mg/kg i.p. EDO 133mg/kg) c-fos levels were highest. A time-course study revealed that after caffeine injection, c-fos levels increased rapidly to reach a maximum at 15-30 minutes and declined progressively to basal levels at 4 hours. The pharmacological profile of subconvulsive caffeine-induced c-fos expression was also investigated, and suggests that both benzodiazepines and adenosinergic mechanisms may be involved in this system.

463.2

TRANSIENT CEREBRAL ISCHEMIA (TCI) INDUCES C-FOS ONCOGENE EXPRESSION IN THE RAT BRAIN. D.H. Gehlert, M.B. Jorgensen, J. Deckert and D.C. Wright. ETB and SBR, NINCDS, N.I.H., Bethesda, Maryland 20892, Neurophysiological Institute, Copenhagen, Denmark and University-Sherbrook, Forth, FRG.

The c-fos protein is a cellular product that appears in neurons rapidly following neuronal stimulation. The c-fos proteins are found in the nucleus and appear to be associated with chromatin. The c-fos protein has been implicated in the cell cycle function in cells in those cases that may occur in learning and memory. The basal levels of c-fos in the brain are very low but are reported to increase rapidly following sensory stimuli. Therefore, we have examined c-fos mRNA in the brain following TCI.

Under anesthesia, the vertebral arteries of male, Sprague-Dawley rats were cauterized and the common carotid arteries were isolated and exposed. Twenty-four hours later severe forebrain ischemia was induced by occluding the common carotid arteries. Animals were killed at various time periods thereafter from 3 to 72 hours. The brains were removed and sectioned at 20 microns. Following formaldehyde post fixation, the sections were hybridized using a riboprobe labelled with 35S-nucleotides (Lofstrand, Gaithersburg, MD). Hybridization was detected by autoradiography using sheet film or Kodak NTB-2 emulsion.

TCI resulted in a dramatic increase in hybridization for c-fos mRNA in the brain. In general, the expression followed the distribution and time course of TCI-induced damage in the brain. High levels of expression were seen in individual neurons of the caudate-putamen, CA1 of the hippocampus and in scattered neurons in the hilus of the area dentata. These results indicate that c-fos may play a role in the damage and recovery of brain neurons following TCI.

463.3


The proto-oncogene c-fos encodes a nuclear phosphoprotein, which is thought to act as a transcriptional factor for other specific genes. c-fos gene expression is readily detectable in adult mammalian brain and is extremely inducible in neurons by seizure activity, cholinergic receptor agonists and depolarization. To examine the in vivo effects of adrenergic drugs on c-fos mRNA expression in brain, adult male rats were injected ip as follows: 1.) vehicle (V) alone; 2.) yohimbine (Y) (α2 antagonist) alone; 3.) clonidine (C) (α2 agonist), propranolol (P) (β antagonist) or prazosin (Pz) (α1 antagonist), alone or preceding yohimbine injection. We observed a transient induction of c-fos mRNA following vehicle injection, which was enhanced and prolonged by Y injection. Either C or P suppressed the vehicle induction, while C, P, and Pz suppressed induction of c-fos mRNA by Y. These data are consistent with the hypothesis that c-fos mRNA levels increase as a result of the interaction of norepinephrine (NE) with the post-synaptic α2-adrenergic receptor. According to this interpretation, the effects of Y and C would be due to modulation of NE release by the autoradiographic effect of the pre-synaptic α2-adrenergic receptor. Supported by NIH/NS36820 and Colleen Giblin Foundation.

463.4

LOCALIZATION OF INCREASED c-fos mRNA CONTENT IN RAT CNS FOLLOWING RECURRENT SEIZURES. Christine Galt, Amy Arac, and Jeffrey White, Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717 and Div. of Endocrinology, SUNY, Stony Brook, NY 11794.

The product of the cellular proto-oncogene c-fos has been suggested to be a regulatory factor in gene transcription. Recently, several groups, including ourselves, have demonstrated that c-fos protein and/or mRNA content in the CNS is increased in response to seizures. In the present study, in situ hybridization autoradiography was used to evaluate the correspondence between changes in the abundance of mRNAs for c-fos and preproenkephalin A following seizure activity.

Blistered recurrent seizures were induced by either unilateral electrolytic lesion of the dentate gyrus hila (HL) or parietal path stimulation in anesthetized male rats. Pertinent fixed tissue sections from forebrain of experimental and paired control rats were processed for the localization of c-fos mRNA using a 35S-tRNA probe. Within 15min. of electrically stimulated seizure initiation, hybridization was increased exclusively within the dentate gyrus granule cell layer, bilaterally. At 3hrs post-HL, hybridization to c-fos mRNA was reliably greatly elevated within dentate gyrus stratum granulosum and layer II of perirhinal and piriform cortices. Most increases in hybridization were also generally evident throughout hippocampal stratum parasitale, olfactory tubercle, and anterior olfactory nucleus. By 6 hrs post-HL, hybridization density had declined somewhat in stratum granulosum whereas labeling of piriform cortex had diminished from the 3hr time point. These data indicate an extremely rapid increase in the abundance of c-fos mRNA in most, but not all, areas in which preproenkephalin A mRNA becomes elevated in the same paradigm. The data are consistent with c-fos gene activation in response to seizures but further work, including examination of additional time points, is needed to determine if c-fos transcription is elevated in all areas which exhibit seizure-induced increases in enkephalin synthesis. Supported by NMS 8417068 and R01 NS00915 to CG and MH 42074 to JW.
REGIONAL DISTRIBUTION OF EPIDERMAL GROWTH FACTOR mRNA IN THE MAMMALIAN CNS SYSTEM. R. K. LINCH, M. L. ROBERTS, AND M. BLUM. (SPON: David E. Wolffe, Fishberg Center for Neurobiology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Epidermal growth factor (EGF) is a mitogenic polypeptide, originally isolated from the male mouse submaxillary gland, which has been found to exert a neurotrophic effect on primary neuronal cultures. Recent investigations into the localization of EGF within the mammalian central nervous system have identified regions of mouse and rat brain which contain relatively high levels of EGF-immunoreactive material. While such demonstration of EGF in the CNS might reflect protein sequestration and not brain-specific protein synthesis, another study utilizing dot-blot analysis has demonstrated mRNA for EGF in several brain regions under preparation suggesting local CNS synthesis. Our studies use a more sensitive RNA quantitation assay to focus on the regional distribution of EGF-specific mRNA within the central nervous system of the adult male mouse in order to further explore the brain-specific expression and potential neurotransmitter-neuromodulator functions of this neuropeptide. Using a solution hybridization S1 nuclease protection assay, we have been able to detect as well as quantitate the levels of EGF-specific mRNA in several brain regions of the adult male mouse. Our preliminary studies reveal EGF-specific mRNA at levels of approximately 50 fg per ug total RNA in mouse cerebellum and striatum with no detectable EGF mRNA in cortex. We are currently investigating the developmental pattern of expression in these tissues.

TRANSFORMING GROWTH FACTOR BETA IN BRAIN TUMOR BIOLOGY. Barry T. Meyer and Harold L. Moses. Deps. of Neurology and Cell Biology, Vanderbilt Univ., Nashville, TN 37232

We studied transforming growth factor beta (TGF-beta) in several clones of a virally induced canine glioma brain tumor cell line differing in their ability to produce brain tumors in vivo after intracerebral inoculation of the cells into dogs. In vitro colony formation and TGF-beta secretion by each clone correlated with in vivo tumorigenicity. In vivo tumor growth was documented pathologically in athymic nude mice (immunodeficient) as well as normal nude and normal dogs. The brain tumor cell clones were able to produce brain tumors in normal dogs, however brain tumors were produced in all nude mice and nude dogs pretreated with cyclosporin. Recent data (SMBO Jnl. 5:1633, '87) suggests that TGF-beta-1 or -2 is synthesized by human glioblastoma cells in vivo, contributing to impaired immunoreactivity and allowing brain tumor growth. We thus performed Northern blot hybridization on each clone to quantify POL A mRNA for TGF-beta-1 and beta-2. The jaw tumorigenicity of each brain tumor cell clone correlated with the amount of mRNA for both TGF-beta-1 and beta-2.

We postulate that TGF-beta stimulates glioma cell growth and inhibits host antitumor immune surveillance. Supported by American Cancer Society Clinical Oncology Career Development Award No. 87-118 and NCI RSGC Award No. RR-05432.


In the primary brain the substantia innominata (SI) contains a group of magnocellular neurons, the majority of which are cholinergic. As such, this brain region represents a favourable source of material to characterize the genes expressed by these central cholinergic neurons. In order to examine gene expression in this region, a recombinant cDNA library has been prepared in the plasmid vector pGEM using SI polyadenylated RNA obtained from a neurologically normal individual. The small amount of SI RNA that can be obtained is a major difficulty in differential library screening. After sequencing the cloned genes, the SI library was initially screened with cerebellar cDNA. Clones which did not generate a hybridization signal with highly labelled cerebellar cDNA were further investigated by constructing the CDNA insert for brain regional hybridization analysis using RNA prepared from human SI, cerebellum, neocortex, corpus callosum and caudate nucleus. Clones have been identified which correspond to genes differentially expressed in these brain regions. The sequence and cellular localization of the corresponding mRNAs are under investigation. Supported by a grant from the ADRA, Inc., Chicago. Brain tissues were obtained from the Alzheimer's Program, which is supported by the MRC of Canada and the B.C. Medical Services Foundation.


Cal/mmodulin-dependent protein kinase (CaM kinase II) is a multifunctional enzyme consisting of noncovalent dimers of two subunits, alpha (50k) and beta (65k). Although this kinase is highly concentrated in brain tissue, immunocytochemical analyses have shown that its distribution among different brain regions is highly variable. We have recently shown that the two subunits are expressed at different times and rates during rat forebrain development. In the present study, we have extended earlier findings to the mRNA level. Using oligonucleotide probes specific for each subunit we have examined changes in subunit-specific mRNA levels and their distribution during rat brain development.

While the level of alpha subunit protein is barely detectable at postnatal day 5, its mRNA is already prominent at 4 days and exhibits a distribution similar to that described for the protein in adult brain. The strongest hybridization was associated with the hippocampal formation and olfactory bulb/cortex. The neocortex and various basal forebrain and diencephalic nuclei also showed significant hybridization, which increased with postnatal age. The cerebellum displayed alpha subunit mRNA primarily in the Purkinje cell layer. Hybridization to beta subunit mRNA was also strongest in hippocampal and olfactory structures, followed by neocortex and basal forebrain nuclei. Unlike alpha subunit, hybridization to beta subunit mRNA in neocortex decreased with increasing age, and in cerebellum, was detectable in the granule as well as molecular layers. Our results support previous findings indicating that the expression of the two CK-II subunits are differentially regulated during rat brain development.

DIFFERENTIAL REGULATION OF G-PROTEIN mRNA BY ADRENALECTOMY AND CORTICOSTEROIDE TREATMENT IN RAT CEREBRAL CORTEX. K. S. BOSAK, M. L. SULLIVAN, D. CORDOVA, R. A. COOK, H. T. WHEELAN and H. L. MOSES. Deps. of Neurology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Previous studies have demonstrated that corticosterone (CORT) or adrenalectomy (ADX) alters the adrenergic receptor-coupled cyclic AMP generating system in the brain. The biochemical alterations responsible for these changes could involve alteration of any of the components of the system, such as the receptors, guanine nucleotide binding proteins (Gα, Gβ and Gγ), and the catalytic unit of adenylate cyclase. In the present study, we examined the influence of ADX and CORT on G-protein subunits by measuring mRNA levels for Gαs, Gαi and Gβγ by hybridization blot analysis using specific cDNA clones for each subunit. In addition, the total amount of Gαs, Gαi and Gβγ subunits was determined by immunoblotting analysis.

Gαs mRNA levels in cerebral cortex were increased by ADX, and CORT replacement reversed this effect; in addition, chronic CORT administration alone increased Gαi mRNA levels. Gαs mRNA levels were increased by chronic CORT and a corresponding increase in Gαi immunolabeling was also observed; in addition, ADX tended to decrease Gαi mRNA levels. Gβγ mRNA levels also tended to increase after CORT, while Gαs mRNA levels and immunoreactivity were not altered by ADX or CORT treatment.

The results represent the first demonstration of differential regulation of G-protein mRNA levels and immunolabeling in the nervous system. These alterations of G-protein subunits may in part account for the influence of glucocorticoids on the adrenergic receptor-coupled cyclic AMP system in brain.

PHORBOL ESTERS STIMULATE APPEARANCE OF ASYMMETRIC ACETYLCHOLINESTERASE IN TTX-TREATED QUAIL MUSCLE CULTURES. G. FERNANDEZ-VALLET and R. A. BORTOLAZZO. Dept. of Anatomy and Cell Biology, Univ. of Miami, Sch. of Med., Miami, FL 33101

The major acetylcholinesterase (AChE) forms synthesized by tissue-cultured quail muscle are globular monomers, dimers and tetramers and asymmetric molecules, composed of three tetramers covalently linked to a collagen-like tail. Inhibition of spontaneous muscle contraction by tetrodotoxin (TTX) prevents appearance of asymmetric AChE, the predominant enzyme form at the neuromuscular junction. This does not occur via changes in synthesis, activation or degradation of newly-synthesized AChE polypeptide chains, but rather through a 2-fold increase in the fraction of total AChE activity secreted by non-contracting muscle cultures. This is suggestive of a block in assembly of tetramers with collagen-like tail subunits in the Golgi apparatus. We have found a role for increased protein kinase C with phorbol esters stimulates the appearance of asymmetric AChE in TTX-treated cultures. Inhibitors of PIP2 hydrolysis decrease this activity to levels observed in TTX-treated cultures without affecting contractile activity. These studies suggest that regulation of synaptic components may occur through activation of second messengers in response to membrane depolarization rather than contractile activity per se. This work was supported by grants from the NDA and NIH to RS; CPV is a State of Florida Pre-doctoral Fellow.
EXPRESSION OF MULTIPLE ACETYLCHOLINESTERASE TRANSCRIPTS IN QUAIL MYOTICES IS REGULATED BY PATERNAL ACTIVITY. M. A. Randall*, C. Fernandez-Valle*, and R. L. Rutendo (SPON: A. Boyne). Department of Anatomy and Cell Biology, Univ. of Louisville, School of Medicine, Louisville, Kentucky 40292.

Multiple oligomeric forms of acetylcholinesterase (AChE) expressed in avian and Torpedo nerves and muscle are encoded by a single gene. Using a dimeric cDNA probe (Schumacher et al., Nature 310: 407, 1986) we have isolated cDNA clones encoding quail AChE catalytic subunits. The identity of one cDNA clone was confirmed by DNA sequence analysis. Comparison of deduced amino acid sequences of Torpedo and quail AChEs so far obtained shows greater than 90% identity. One cDNA (Mr 44,500) is large enough to encode the entire open reading frame. Northern blot analysis of quail brain and muscle poly A+ RNA indicates the presence of multiple AChE transcripts in the range of 4.8 to 6 kb. Quail myoblasts express several AChE transcripts and synthesize membrane-bound and secreted AChE forms. The levels of AChE mRNAs, as well as rates of AChE translation, increase following myoblast fusion and subsequently decrease with the onset of spontaneous muscle contraction. Treatment of spontaneously contracting myotubes with tetrodotoxin results in a large increase in AChE mRNA. These studies show that expression of the AChE gene is developmentally regulated as well as under control by the activity state of the muscle. Supported by grants from the NIH and MDA to EKR.

APPENDIX A

THURSDAY PM


Brainstem trigeminal vascular convergence (TVC) neurons receive an excitatory, nociceptive input from cranial blood vessels as well as the periorbital skin or cornea. TVC neurons that responded to electrical stimulation of the superior ophthalmic vein or ophthalmic artery as well as the cornea were identified with intracellular recording and then labelled with HRP. TVC cell somata were localized to the lateral part of lamina V in the rostral trigeminal nucleus caudalis; this distribution extended anteriorly into nucleus interpolaris, and ventrally into the trigeminal extension of the lateral reticular nucleus. Labelled neurons were typically either fusiform, with the soma and dendrites oriented along a ventromedial to dorsal-lateral axis, or multipolar in shape. Two patterns of TVC axonal projections were observed. The first type ascended through the spinal trigeminal complex, contributing multiple collaterals with terminations in lamina V and the adjacent medullary reticular formation. The second axon type crossed the midline to ascend in the trigeminothalamic tract. Before crossing the midline, some such axons collateralized in the dorsomedial reticular formation at the borders of the vagal and solitary nuclei. This pattern of terminations supports a role for TVC neurons in both sensory and autonomic cerebrovascular function.


To determine the morphological features of functionally identified V neurones, HRP-filled micropipettes were used to record and characterize the physiological properties of single neurones within V subnuclei oralis and adjacent brainstem regions. HRP was then injected intracellulary and the brainstem perfused. The 8 labelled oralis neurones had a mechanoreceptive field localized within one V division and short-latency (2-6 ms) electrically evoked inputs from trigeminal spinal neurones in both oral and incisive branches. The somata of these neurones were located in the mediodorsal aspect of lamina V and adjacent medullary reticular formation. The soma diameters averaged 7-8 μm and extended beyond the borders of the subnucleus. Many of these neurones contrasted with those of functionally identified neurones of the V subnucleus to the region N issued from V subnucleus. Many of these features contrasted with those of functionally identified neurones of the V subnucleus.

Response was found to single unit activity in the thalamus using glass coated tungsten electrodes. In some cases traction on the vessels was used to test for mechanical activation while in others blood flow at 100-150 ml/min可通过机械刺激在某些情况下使用来测试。Electrical stimulation activated 44 units of which 23 were in VMH. Fifteen of these responded to SS stimulation, 5 to MMA and 3 to both. The mean latency to SS and MMA stimulation were found in the region of the posterior group of the thalamus. Some of these had face fibers but they were often broad and sometimes bilateral. Seven responsive units were located in the zone incisurae although one was in the interlaminar complex.

464.4 SINGLE UNIT RESPONSES OF INFERIOR DENTAL NERVE TO THERMAL STIMULATION OF THE MOUTH. T. H. Correll*, T. K. Hara*. Dept. of Oral Pathology, School of Dentistry, University of Buffalo, Buffalo, NY.

CGRP-LI was found in the brain stem trigeminal subnuclei. CGRP-LI is found in the spinal trigeminal ganglion and all central sensory nuclei except the mesencephalic nucleus.


464.6 CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY IN FELINE TRIGEMINAL NUCLEI. L.A. Jones², M.A. Henry², L.E. Wustrum². Dept. Physiol., Univ. of Colorado, Denver, CO 80262.

Recent studies have localized calcitonin gene-related peptide (CGRP) in the central nervous system. The aim of this study was to determine the CGRP-LI in the trigeminal nuclei of the cat. CGRP-LI was found in the spinal trigeminal subnuclei. CGRP-LI is found in the spinal trigeminal ganglion and all central sensory nuclei except the mesencephalic nucleus. In the main sensory nucleus (MSN) CGRP fibers in the ascending tract send a dense projection to the dorsal horn of the spinal trigeminal nucleus. In the intermediate nucleus (MN) CGRP fibers show a sparse projection to the sensory areas of the spinal cord. The relationship between CGRP-LI and thermal, mechanical and chemical stimulation of the tooth, firing rate of the fibers were correlated to the subjective sensation of the tooth, although after discharges continued.
PAIN PATHWAYS: TRIGEMINAL SYSTEM


Jacquin, this volume), but the arbors most often are large and could contain completely one of these PrV cells.


A neuron reconstruction system (Buteric Electra) was used to quantify the 3-dimensional structure of 2 electrophysiologically characterized, HRP-labeled, primary afferent fiber collaterals in the "��" region of rat principalis (PrV). Each responded phasically to deflection of 1 mystacial whisker and gave rise to 6 collaterals with arbor distributed discontinuously in topographically appropriate regions of PrV.

Prior retrograde tracing and Golgi studies, primarily in cat, indicate a complex web of intersubnuclear connections within the trigeminal (V) brainstem complex. In rat, HRP-stained local circuit neurons, and some projection cells, in subnucleus caudalis, interporalis, and oralis, have been shown to project to rostral and caudal subnuclei. In the present study, anterograde transport of Phaeolus vulgaris leuco-agglutinin has allowed us to demonstrate that the patches observed with other methods indeed terminal arborization. Labelling of individual fibers by means of intra-axonal injection of horseradish peroxidase demonstrated that individual trigemino-tectal axons contributed to several patches across the mediolateral extent of the stratum album intermedium. We also noted that the SC projection from PrV consistently had several fibers which recrossed the midline in the SC commissure to give rise to patches in the rostral subnucleus in the SC ipsilateral to the injection site. Supported by BNS 85-00142, BNS 85-15737, EY 04170, DE 07734.


The intermediate grey layer of the superior colliculus in the rat contains projections from the spinal trigeminal nucleus. The present experiments were designed to characterize the terminations of this pathway as a step toward determining their contribution to the output of the various efferent pathways of this layer. We placed electrophoretic injections of 2.5% biotinylated PHA-L (Vector) in subnucleus interpolaris and reacted in diaminobenzidine. For electron microscopy, tissue was embedded and areas containing terminations were thin-sectioned. PHA-L positive fibers are restricted to the rostral two-thirds of the superior colliculus. In the intermediate grey layer (Weiner's nomenclature), the label appears "patchy" because of variations in terminal and fiber density. The labeled axons are numerous laterally, and become sparse medially. Most axons are fine and highly branched, giving the appearance of boutons en passant. Electron microscopic examination reveals that labeled terminals are filled with densely packed, small round vesicles and contact dendrites.

Experiments are in progress to identify the target cells of these axons. Supported by Grant BNS-86-07060.


A neuron reconstruction system (Buteric Electra) was used to quantify the 3-dimensional structure of 2 electrophysiologically characterized, HRP-labeled, primary afferent fiber collaterals in the "��" region of rat principalis (PrV). Each responded phasically to deflection of 1 mystacial whisker and gave rise to 6 collaterals with arbor distributed discontinuously in topographically appropriate regions of PrV. Excluding this collateral, the remaining 11 collaterals had transverse areas of 1533±1626 mm^2 and volumes of 19852±11507 mm^-3, while arbor limits were 1546±1546 and 724±724 in X,Y,Z respectively. However, one of these collaterals was unusual in having 2 second-order branches which arborized in nonoverlapping regions of PrV. Excluding this collateral, the remaining 11 collaterals had transverse areas of 1188±5187 mm^2 and volumes of 198052±11507 mm^-3, while arbor limits were 1546±1546 and 724±724 in X,Y,Z respectively. The arbors the remaining 11 collaterals had a similar geometry to that of single whisker sensitive, thalamic-projecting cells in PrV (Golden & Jacquin, this volume), but the arbors most often are large and could contain completely one of these PrV cells.


Prior retrograde tracing and Golgi studies, primarily in cat, indicate a complex web of intersubnuclear connections within the trigeminal (V) brainstem complex. In rat, HRP-stained local circuit neurons, and some projection cells, in subnucleus caudalis, interporalis, and oralis, have been shown to project to rostral and caudal subnuclei. In the present study, anterograde transport of Phaeolus vulgaris leuco-agglutinin was used to visualize the projections and morphologies of V intersubnuclear axons. Injectiions restricted to caudalis (N=4) resulted in dense terminal labeling in each of the more rostral ipsilateral V subnuclei and cervical dorsal horn, and sparse label in contralateral caudalis. Interporalis (N=4) projected heavily to ipsilateral caudalis, oralis and principalis. Principalis (N=4), on the other hand, had only a sparse projection to each of the caudal ipsilateral subnuclei. The smaller of the above injections showed axon endings in regions topographically matching their sites of origin. Individual collaterals could be reconstructed in most cases. Their morphologies were as previously described for single HRP-stained cells. Axons traveled within the deep bundles of the V brainstem complex, the V spinal tract and reticular formation. Most collaterals gave rise to circumcrosed and highly branched arbors with a large number of terminal and en passant boutons. Supported: NIH DE07662, DE07734, NSF NINS515737.
SUBCORTICAL SOMATOSENSORY PATHWAYS: TRIGEMINAL SYSTEM

THURSDAY PM


A neuron reconstruction system (Kutetuc Elect.) was used to quantitate the 3-dimensional structure of 5 HF-labeled cells in the "barrel" region of rat principalis. Each responded phasically to 1 mystacial whisker and projected only to that branch. Mean latencies to V ganglion and tactile shocks were 1.1 and 1.4 ms. Small somata gave rise to 5.6 responses to 1 mystacial whisker and projected ± points. All dendritic branching occurred within 100um of the soma, with the largest # of branch points between 20-30 um. 64±2 dendritic appendages (swellings, spines) were equally distributed across branch order and were most numerous on 0.4-1.2um thick branches. Total dendritic length, surface area and volume were 884K, 2740K, 59um2 and 925 ±293um3, respectively. Dendritic tree envelopes had transverse areas of 3235±863um2 and volumes of 2972±1047um3. Each tree was polarized, spanning no greater than a hemisphere around the soma; however, there was no reliable direction of polarity. Centers of dendritic area, relative to somata, were 45±2, 2±8 and 5±1um in X,Y,Z, respectively. Tree limits were 68±4, 95±4 and 91±29um in X,Y,Z, respectively. These small and dense dendritic trees have similar shapes to that of whisker afferent collaterals in principalis (Panneneton et al., Soc. Neurosci. Abst.; this volume). Their matching shapes may explain receptive field size in single-whisker principalis cells. Support: DB07662, DE07734.


Intracellular recording, electrical stimulation, receptive field mapping, and HRP injection techniques were used to study principalis cells in rat. 80 cells provided physiological data. They responded within 1.2±0.2 ms of trigeminal ganglion shocks and 65% were antidromically activated by thalamic shocks (1.3±0.5 ms latencies). 69% were whisker-sensitive; of these, 80% were responsive to 1 whisker (mean±1.5, range=1-8), were slowly adapting, 40% were direction sensitive, 35% were spontaneously active, 2X had an inhibitory surround, and 42% also discharged to guard hairs. The test response (mean±SD) was 1.11±0.39, and had max. (1.3), min. (5%), teeth (12%), and nociceptors (8%), and were unresponsive. 17 cells were HRP-stained. Thalamic-projecting cells with 1 whisker, guard hair, or skin receptive fields (N=12) had small somata and dendrites which extended only a short distance from the soma, where they branched extensively. Spheres were rare, yet swellings were common. Axons never branched locally. Within this group, slowly adapting cells tended to have bigger dendritic trees with more swellings. As a group, however, these 12 cells had very different morphologies from those of nociceptive, and 2 multi-whisker projection cells, and 2 local circuit cells. Larger somata gave rise to expanded and spiny dendritic trees, with local, interneuronal, and reticulax axon collaterals. These data are suggestive of structure-function correlations in principalis. Support: NIH DE07662, DE07734.


Intracellular recording, electrical stimulation, receptive field mapping, and HRP injection techniques were used to study form-function correlations in oralis of the rat. Of 15 labeled cells, 4 were local circuit neurons responsive to either an incisor, guard hair, 1 vibrissa, or deep pressure. Dendritic morphologies most closely resembled those of V-thalamic cells in subnucleus interpolaris (Jaquint et al., Exp. Brain Res.; 91:160); their axons had interneuronal collaterals. Thalamic- (N=6) and cerebellar-projecting (N=2) cells had response properties and morphologies which were similar to interpolaris cells with equivalent projections. 2 cells projected to spinal cord, as well as other V subnuclei; one responded to strong pressure applied to an incisor; the other was a whisker sensitive. Their morphologies did not differ from other oralis and interpolaris projection neurons. The remaining cell had 2 axons, one projecting to thalamus, the other to spinal cord. One responded to 15 vibrissae, guard hairs and glabrous skin. Its somadendritic morphology was similar to that of other oralis projection neurons. The extensive dendritic trees, local and long-range axon branching, multi-whisker convergence, and functional diversity of oralis cells approaches that observed in interopolaris. Such anatomical and physiological properties are rarely seen, however, in subnucleus principalis. Support: NIH grants DE07662 and DE07734.


The inner conal body (ICB) in a mystacial vibrissal facial SC is heavily innervated only in species that whisk (Rice et al., JCN 252:158). As seen in LM silver preparations of the adult rat ICB, numerous receptors are parallel to each other and encircle the vibrissal follicle in a plane perpendicular to the hair shaft. One of our goals was to determine the ultrastructure of the adult rat ICB innervation by standard TEM of thin sections and High Voltage EM of thick (0.25-0.5um) sections. Numerous endings were observed: many nearly free of surrounding structure (free nerve endings?) of which one novel type resembled gunshot; many embedded in thick collagen matrices (?); some affiliated with collagen fibers in a "septal cell" capsule (Ruffini endings?); and a few others flattened between two satellite cells (lameolate endings?). Another goal was to evaluate some neuropeptide 1M immunoreactivity (IR) within the ICB. Numerous, but clearly not all processes expressed substance P- and COR-like IR. No ICB processes expressed somatostatin-, VIP-, or NPY-like IR. (Support: Swedish Medical Research Council and NIH PBS RO1219 to Drs. Donald Parsons and Min Song, NY State HVEM Facility)

465.9 PRINCIPALIS- OR PARABRACHIAL-PROJECTING SPINAL TRIGEMINAL NEURONS DO NOT STAIN FOR GABA OR GAD. J.R. Hourig & M. F. Jaquint. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Most low-threshold, mechanoreceptive local circuit neurons in rat spinal trigeminal (SpV) subnuclei interpolaris and caudalis have collaterals which end in more rostral subnucleus caudalis (PvC). It has been hypothesized that these cells have GABA-ergic processes which serve to gate transmission of V primary afferent inputs to projection neurons in SpV and PvC. Retrograde transport of dianodino yellow (Dyo) and immunohistochemical double-labeling procedures were used to determine if pontine-projecting SpV cells stain positively for GABA or GAD. As expected, large bilateral injections of Dyo into rostral and lateral pons (N=10), inclusive of LV and parabrachial nuclei, labeled large #s of cells in each SpV subnucleus. Brainstems (2 collicits pretreated) were subsequently reacted for cytoplasmic GAD or GABA immunoreactivity (Weinberg et al., Neurosci. Lett. 55:349, '85). A large # of cells throughout SpV were immunopositive for GAD. 400, 200 and 160 cells per cross-section through caudalis, interpolaris and oralis, respectively, with the heaviest concentration in ventral interpolaris and lamina II of caudalis. However, none were double-labeled with Dyo. This result does not support the above-stated hypothesis, suggesting that SpV local circuit neurons with PVr collaterals are not GABA-ergic. These studies also indicate that parabrachial-projecting SpV cells are not GABA-ergic. Support: NIH DE07662, DE07734, NS25752.
CHEMICAL SENSES: PERIPHERAL MECHANISMS II


Cyclic AMP is an attractant stimulus to paramecia. As other attractants, it hyperpolarizes the cells and shows specific, saturable, albeit low affinity binding to whole cells or selectively to cilia on the cell membrane. A 48 Kd protein identified by affinity chromatography is a candidate for the cAMP receptor, as judged by its specificity for binding to the column. Although similar in molecular weight, it is not identical to the regulatory subunit of the Paramecium cAMP-dependent protein kinase on Western blots (antibody provided by M. Hochstrasser, 26). In 29 electrophoresis, the 48 Kd protein is extreme in its pi (2.5). Con A-BP does not appear to bind to the protein; however, it still may be a glycoprotein. Since the N terminus is blocked, microsequencing is being carried out on CNBr fragments. The sequence will be used to produce an oligonucleotide probe for cloning the receptor gene. Polyclonal antibodies have been produced in New Zealand rabbits against gel slices containing the antigen. These antibodies recognize the antigen in mg amounts on slot blots and, therefore, are appropriate for screening an expression library of lambda gill for the receptor gene. Supported by NSF.

466.2 FOLATE CHEMORECEPTOR MUTANT: ANALYSIS OF SURFACE MEMBRANE PROTEINS IN PARAMECIUM. J.W. Saaser, J.K. Isachsen, and J.L. Van Houten. Zoology Department, University of Vermont, Burlington, VT 05405.

Paramecia are attracted to folate by a chemokinetic mechanism. The cell body membrane binds folate specifically and saturably (not cilia), and this binding correlates with the change in membrane potential that determines swimming behavior. We are trying to identify the receptor protein for folate by comparing the folate-binding proteins of membrane preparations of wild-type cells with those of mutants that do not normally bind or respond to folate.

We have developed a method to covalently crosslink folate to cells that specifically blocks folate chemoreception, and have developed an antibody that specifically detects the ligand. Preliminary data also shows that we can covalently crosslink folate to membrane preparations. We are using these techniques to identify membrane folate binding proteins and by comparison of wild-type and mutant folate binding proteins, will identify the chemoreceptor protein.

Supported by NSF and Whitall Foundation.


We are studying neurotransmitter and transduction mechanisms in a chemosensory organ, the rat carotid body, using dissociated cell cultures (see accompanying abstract, A. Stea and C.A. Nurse). Previous studies in this laboratory indicated that single (parenchymal) glomus (type I) cells in these cultures may express both catecholamine histofluorescence as well as acetyleholinesterase (Cell Tissue Res. 212:257). We now show by the use of immunofluorescence techniques that these glomus cells selectively express both tyrosine hydroxylase and neuron specific enolase in low amounts. In addition, histochemical staining for carbonic anhydrase (CA) indicated a selective localization in glomus cells; staining was markedly enhanced following permeabilization of the cell membranes, suggesting a predominant intracellular localization, and was abolished by the Ca-inhibitor acetazolamide (10 mM). Unstained cells included sustentacular (type II) cells and fibroblasts. This finding is important since CA-localization in the carotid body is controversial and bears on the precise formulation of the acid hypothesis, proposed to explain the organ's response to increased blood CO2 (and H+) levels.

Supported by the Heart and Stroke Foundation of Ontario.

466.4 SURFACE HETEROGENEITY OF HUMAN OLFACTORY EPITHELIUM. M. Zheng and R.J. Tait. Dept. of Anatomy, University of Hong Kong, Hong Kong and Dept. of Anatomy, Jinan University, Guangzhou, China.

We have conducted scanning (SEM) and transmission electron microscopie (TEM) observations on the human olfactory epithelium (OE) of the nasal septum (NS) and superior conchae (SC). Our findings show that the human OE surface, hitherto assumed to be uniform, exhibits considerable heterogeneity.

In the SC, the OE surface was highly ciliated, suggesting a high density of receptor cells. For the most part, the length of the cilia was uniform. Microvilli, indicating the presence of supporting cells, were generally present in small pockets. In SEM, dendrites were observed to give rise to large numbers of cilia, more numerous than previously estimated from TEM studies. However, the usual knob-like endings of the dendrites were not apparent.

In the NS, the distribution of ciliated and microvillous surfaces was more variable than that of the SC. Generally, the ciliated regions occupied a smaller area; and patches of cilia of different lengths were often present in close proximity. Some dendrites bore radiating arrangements of cilia, which were not observed in the SC. TEM observations of young receptor cells showed that many of them possessed cilia with expanded tips (primary cilia?). Ductal openings of Bowman's glands were more numerous in the NS.

466.5 NUMERICAL RELATIONS BETWEEN OLFACTORY RELAY ELEMENTS (RECEPTOR NERVES; GLOMERULI AND MITRAL CELLS) IN THE CAT. L.J. Smart and L. McComb. (SPON: C.L. Prosser). Dept of Physiology, Univ. Illinois, Urbana, IL 61801.

The olfactory mucosa (OM) and bulb (OB) of adult cats were analysed quantitatively for the number of primary olfactory neurones (ON), the glomeruli (GL) and mitral cells (MC) using light microscopic, numerical and morphometric analysis. An empty fronted series of 10 um whole sections stained with H&E and Nissl were employed. The results indicate that the cat possesses, on each side of the nasal cavity, about 40 million MC with convoluted cilia onto about 40,000 MCA in each OB. This gives an ON/OC convergence ratio of about 1000:1, the same as in the macromammals. The MCs, categorized as a finger-like endings of the main glomerular diameter was taken as 70 um (i.e., mean diameter of all GL per section) or 3300 if the mean diameter was taken as 105 um (i.e., average of the largest GL diameters per section). The OB volume is about 50 cu mm and MC layer surface area about 50 sq mm giving an average of 5000 sq mm. Total surface area is 600 sq mm 20% covering the septum 80% the choana; the surface density of the ONs being about 70,000/sq mm. The ONs are about 50 um thick and have 9 and 3 ONs for each basal and supporting cells respectively.

466.6 LIQUID ODORANTS DELIVERED TO THE VOMERONASAL ORGAN OF GARTER SNAKES INCREASE FIRING OF CELLS IN THE ACCESSORY OLFACTORY BULB. A. Iwanicki, J. E. Kuhls, and M. Hildebran. Dept of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. 11203.

Most terrestrial vertebrates possess both main olfactory and vomeronasal systems with many features in common. There have been few studies using electrophysiological techniques to characterize and compare the properties of these two systems. To examine the physiological properties of both systems in garter snakes (Thamnophis sirtalis parietalis), we first focused on recording electroolfactograms (EOGs) from the olfactory and vomeronasal epithelia. Using airborne odorants we were able to elicit EOGs from the olfactory but not vomeronasal epithelium although the responses from the vomeronasal epithelium were very small (less than 1 mV). It has been suggested that the vomeronasal system is normally stimulated by odorants delivered as liquids. In recordings from individually recorded sensory olfactory bulb neurons we found a variety of liquid odorants, including amyl acetate and earthworm wash, induced dramatic increases in neuronal activity. These results demonstrate that the vomeronasal system is sensitive to a variety of odorants and support the idea that under normal conditions effective stimuli arrive in a liquid medium. Supported by NIH Grant NS17173.
466.7 EOG AMPLITUDE IS CORRELATED WITH ODOR-STIMULATED ADENYLATE CYCLASE ACTIVITY IN THE STRIPED HOG NOSE FROG OLFACTORY EPITHELIUM. Graeme Lowe*, Tadashi Nakamura* and Geoffrey H. Gold. Morrel Chemical Sensors Center, Philadelphia, PA.

Recent work suggests that odor-stimulated adenylate cyclase (AC) activity mediates olfactory transduction in vertebrates (Pace et al., 1985; Shirley et al., 1986; Nakamura and Gold, 1987). However, stimulation of the AC by certain odors is weak or undetectable, leading to the suggestion that the AC may not mediate transduction for all odors (Sklar et al., 1986). An alternative explanation for these data is that differences in the magnitude of AC stimulation by various odors may reflect differences in the number of receptor proteins or receptor cells sensitive to those odors. If the latter hypothesis is correct, we expect the amplitude of the EOG to be correlated with the magnitude of AC stimulation by various odors.

The EOG was recorded from excised bullfrog olfactory epithelium mounted in a perfusion chamber. Odors were applied to the apical side, dissolved in Ringer's solution at a concentration of 100 μM to approximate the conditions used in Sklar et al.'s cyclic assay. Of 35 odors tested thus far, 31 odors elicited a monophasic negative EOG, and 4 odors elicited a multiphasic negative EOG. The multiphasic responses may contain contributions from currents unrelated to transduction, and therefore, were not included in the analysis below. The correlation coefficient relating the peak amplitude of the EOG to the magnitude of AC stimulation for each odor was 0.87. This suggests the ability of odors to stimulate adenylate cyclase is correlated with their ability to generate an EOG response. These results support the adenylate cyclase model of olfactory transduction.

466.9 A SUBSET OF RAT PRIMARY OLFACTORY NEURONS AND GLomeruli SHOWN BY ANTIBODY TO HUMAN PLACENTAL HUMAN PLACENTAL WAP COMMON TO ESTROGEN-BIOSYNTHETIC ORGANS. K. Shinoda**, Y. Shiotani**, J. Pearson and Y. Osawa** . 1. Osaka University Medical School, Japan, 2. NYU Medical Center, NY, 3. Medical Foundation of Buffalo, NY.

Primary olfactory neurons have not yet been extensively characterized, and a polyclonal "modified glomerular complex" located caudally between the main and accessory olfactory bulbs is involved in "nipple licking" behavior. Response to nipple and amniotic fluid odors in rats. We now report localized immunoreactivity in frozen sections of paraformaldehyde-fixed rat olfactory bulb using an antisera to human placental antigen. The antiserum stains efferent synaptic terminal organs (including placenta and ovary) and suppresses 73% of the activity of human placental P450 aromatase. A distinctive subset of rat olfactory glomeruli forms a necklace-like pattern at the caudal end of the rat olfactory bulb and is associated with a subclass of primary olfactory receptors. Part of this subset of sensory neurons in the rat primary olfactory system appears identical with the glomeruli responsible for suckling behavior.

466.10 CONVOLUCANAL A CYTOCHEMISTRY ON RAPIDLY FROZEN FROG OLFACTORY EPITHELIAL SURFACES FREEZ-ETCHED AND ROTARY-REPULICATED WITH TANNANUM/UMTENSTUGEN OR FREEZE-SUBSTITUTED AND EMBEDDED IN LOWCRYL. K11M. B. PH. M. Menco and T. Baldwin. Department of Biological and Physical. C. O. Hogan Hall, Northwestern University, Evanston, IL 60208.

Recent work suggests that odor-stimulated adenylate cyclase (AC) activity mediates olfactory transduction in vertebrates (Pace et al., 1985; Shirley et al., 1986; Nakamura and Gold, 1987). However, stimulation of the AC by certain odors is weak or undetectable, leading to the suggestion that the AC may not mediate transduction for all odors (Sklar et al., 1986). An alternative explanation for these data is that differences in the magnitude of AC stimulation by various odors may reflect differences in the number of receptor proteins or receptor cells sensitive to those odors. If the latter hypothesis is correct, we expect the amplitude of the EOG to be correlated with the magnitude of AC stimulation by various odors.

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Few cell-type-specific antibodies exist for olfactory epithelial cells. In order to develop markers for olfactory cells that would cross-react with both rat and frog, unfixed nasal tissue homogenates from bullfrogs (Rana catesbeiana) or Sprague-Dawley rats were alternately injected intraperitoneally into a CB6F1/J mouse at two week intervals. Spleen cells from the mouse were fused with X-63-Ag8.65, a mouse plasmacytoma eradicating feeder plates in HAT selective medium and positive clones were chosen by immunocytochemistry on cryostat sections of bullfrog olfactory epithelium.

Supernatant from the 1D9.8B clone demonstrated reactivity for sustentacular cells of bullfrog olfactory epithelium, using indirect immunofluorescence. Frog olfactory neurons and nerve tracts were negative. On 4% paraformaldehyde perfused, cryostat sections of rat olfactory epithelium, 1D9.8B supernatant showed binding to a subset of sustentacular-like cells. No staining of olfactory neurons or nerve bundles was observed. In cell cultures of adult rat nasal tissue, 1D9.8B immunocytochemical staining was observed on cells that were large, polygonal and rare in culture. The 1D9 antigen was internal and associated with the cell membrane. Further characterization is being done.

The monoclonal antibody, 1D9, promises to be a powerful tool and is in use for investigation of olfactory epithelial biology. (Supported by NIH PO1 NS23348-03, NSF BNS8344025 and NIH NS25352.)


Olfactory bulb ablation causes olfactory neuron (OSN) death followed by partial reconstitution of the neuronal population (Costanzo and Grawizelad, 1983). Consequently, the morphological and functional differentiation of OSN's was studied after bulb ablation in adult rats with LM, EM and immunohistochemistry in an attempt to define which of the multiple phenotypic properties require the influence of the olfactory bulb. There are three major findings. First, the OSN's on the ablated side are morphologically "immature" since the olfactory bulb is not essential for growth and development [Farman and co-workers]. Second, the uniquely "juvenile" phenotype of OSN's [vimentin expression, lack of expression of NGF and Thy-1] is independent of the bulb, as is the small subpopulation of OSN's that appear more "mature" and express NGF or Thy-1 expression. Third, the expression of the cell surface position-specific R8-8 antigen is restricted to neurons of the ventrolateral olfactory epithelium and their axons as on the control side and in the case of lesioned OSN's continued presence of the postsymaptic target. Supported by NIH NS 19580 and BRSG 54028.
SPROUTING AND SPROUTING MECHANISMS I

SPROUTING IN THE ELECTROSENSORY LATERAL LINE LOBE OF APTERONOTUS FOLLOWING ABLATION OF PERIPHERAL NERVE BRANCHES

SPROUTING IN THE ELECTROSENSORY LATERAL LINE LOBE OF THE TELEOST APTERONOTUS FOLLOWING ABLATION OF PERIPHERAL NERVE BRANCHES

SPROUTING AND SPROUTING MECHANISMS II
467.11


End plates from extensor digitorum longus (EDL), soleus and diaphragm muscles of 10- and 25-mos rats were examined for the effect of aging on ultraterminal sprouting, a form of terminal sprouting usually associated with muscle denervation. There was a significant age-related increase in the fraction of end plates exhibiting ultraterminal sprouting in the EDL muscle; the number increased from 1.5% to 25.6%. There were comparatively fewer end plates with ultraterminal sprouts (5-9%) in the soleus and diaphragm muscles of 10-mos rats, and this number did not change significantly with age. Sprout length did not exhibit an age-related change in any of the muscles, although sprouts were consistently longer in the EDL. End plates from muscles of 25-mos animals that had been chronically stressed or exercised were also analyzed. These extrinsic factors did not alter the number of end plates exhibiting ultraterminal sprouting in any of the muscles. It is concluded that in certain muscles, aging may be accompanied by changes in the relationship between the nerve and muscle that may induce the expression of denervation-like characteristics which are not influenced by extrinsic factors previously shown to affect terminal length. Supported by NIH grants A031972 and A029540.

DEVELOPMENTAL DISORDERS

468.1

PRAGMATIC LANGUAGE SKILLS IN CHILDREN WITH SCHIZOPHRENIA. J. G. Froud and D. G. O. Spanier. Ins. Neuropsychiatric Inns., UCLA, 760 Woodrow Plaza, Los Angeles, CA 90024.

Pragmatic language skills have been found to be impaired in adult schizophrenics (e.g., Rochester & Martin, 1979). The purpose of this study was to investigate the pragmatic skills of children with schizophrenia and to elucidate the nature of these deficits. 15 schizophrenic children aged 7-12 years were matched by sex, MA and SES to normal children. The schizophrenic children were recruited from UCLA's Neuropsychiatric Institute and Outpatient Children's Services and from 2 L.A. schools for the emotionally disturbed. They were independently diagnosed with the Interview for Children's Psychosis (ICPS) by the Diagnostic Unit of the Children's Psychosis Clinic, Language samples were obtained from videotapes of the KF Formal Thought Disorder Story Game (SG) developed by Caplan (1988). The transcribed videotapes were rated with the Pragmatic Skills Scale (PSS) by 2 trained, blind raters. This instrument was adapted from Halliday and Hasan (1976) and operationalized for use with children.

Preliminary results indicate that the schizophrenic children differ from normal children in their use of endophora and unknown ties. The relationship of these behaviors to age and diagnosis will be presented in detail.

468.2


Monoamine oxidase (MAO), in its two forms MAO-A and MAO-B, is the enzyme primarily responsible for degradation of amine neurotransmitters. A complementary DNA (cDNA) clone for human MAO-A has been used to establish the deletion of its corresponding gene in two male cousins with Norrie disease. No MAO-A activity was detected in their fibroblasts. MAO-B activity in platelets and fibroblasts from these patients was also nondetectable. Moreover, major catecholamine metabolites, including vanillylmandelic acid (VMA), homovanillic acid (HVA) and 3-methoxy-4-hydroxy-phenyllglycol (MHPG), were reduced substantially in their urine. These findings indicate that gene(s) necessary for MAO-A and MAO-B activities are deleted in these patients and that the gene loci are near each other in Xp11.3. This is the first report of absent MAO activity in humans; at least some of the clinical features of these patients may reflect this deficiency.

468.3


Our previous studies have shown that pyramidal cell degeneration and monoamine levels are reduced in cerebral cortex of hydrocephalic kittens. The present study sought to determine the relationship between these morphologic and biochemical changes and cerebral blood flow (CBF). Hydrocephalus was induced in 4-10 day old kittens by intracerebral injections of 25% kaolin and verified with ultrasonography. Control animals received similar injections of sterile saline. The isotope labelled microsphere method was used to measure CBF 15-20 days post-injection. Significant (p < 0.05) decreases in regional CBF were detected in the frontal (55%), occipital (52%), parietal (46%), and septum (41). A Cushing response was evidenced by decreases in heart rate (222) and cardiac output (706), and a 211 increase in total peripheral resistance. The changes in cortical CBF did not follow the rostrocaudal gradient of neuronal degeneration and monoamine changes. Thus, these data suggest that reductions in cortical CBF may not be the only factor mediating the neurologic deficits or the neuronal deterioration that accompany hydrocephalus. Supported by NIH Grant # RO105417 to JPM.

468.4

INTERREGIONAL CORRELATIONS OF GLUCOSE UTILIZATION AMONG BRAIN REGIONS IN YOUNG DOWN SYNDROME (DS) ADULTS. B. Horwitz, M. Schapiro, C. Grady, and S. J. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892.

Correlations between regional cerebral metabolic rates for glucose (rCMRglc), determined by positron emission tomography using [18F]fluorodeoxyglucose provide a measure of the functional associations between pairs of brain regions (Horwitz et al., J. Cereb. Blood Flow Metabol., 4, 484-499, 1984). We applied this method to healthy, trisomy 21 DS adults (23-33 yrs) and to 24 age-matched healthy controls (20-35 yrs).

Correlations were obtained between ratios of resting rCMRglc to global brain metabolism (Q-values). Most Q-values had group means that did not differ significantly (p > 0.05) between DS subjects and controls. However, many correlations between regions within and between the frontal and parietal lobes had significantly (p < 0.01) lower values in the DS group than in controls, with some being large and negative in the DS group that were large and positive in the controls. One region so affected was the left inferior frontal gyrus that includes Broca's area.

The decreased values of correlations associated with Broca's region are consistent with the relatively greater language impairment seen in subjects with DS. The reduced values of frontal-parietal correlations in the DS group are similar to that found in adult autistic patients (Horwitz et al., Arch. Neurol., in press), and can be interpreted as indicating a disruption of neural systems associated with directed attention.
468.7

EXPRESSION OF (1) A CELL ADHESION MOLECULE (N-CAM) AND (2) A NEURITE PROMOTING MOLECULE (LAMININ) IN POST-MORTEM BRAIN TISSUE FROM ALZHEIMER'S DISEASE AND DOWN SYNDROME PATIENTS. S-J. Richards & F. Liwii*, Dept. of Biochemistry & Molecular Genetics, St. Mary's Hospital Medical School, London W2 1PG, UK. (Spon. Genentech Laboratory, University of Helsinki, Valmonie 7, Helsinki. 

Down syndrome is a genetic disorder in which affected persons have a karyotype of either trisomy 21 or a translocation of the long arm of chromosome 21, in particular the region 21q22.1-21q22.2. It is the altered expression of the genes within this "pathological region of chromosome 21 that is thought to account for the increased incidence of Alzheimer's disease observed.

Previous behavioral studies have suggested that the increase in genetic material associated with the extra copy or portion of chromosome 21 causes neuronal cytoskeletal abnormalities and which may be causally related to the development of Alzheimer's disease.

We have speculated that the neurological abnormalities may be related to the overexpression of a gene(s) involved in embryogenesis, and further suggest this gene(s) could be a growth factor, cell adhesion molecule or a molecule involved in neural migration and axonal guidance.

To date we have examined levels of expression of N-cam and laminin in post-mortem brain material using Northern blot analyses. We are unable to report a significant variation in expression of either of these two genes within control and experimental brain tissues. (Spon. R.C.A. Pearson)

468.9


Caprine β-mannosidosis, an autosomal recessive defect of glycoprotein metabolism associated with a selective loss of tissue and plasma lysosomal β-mannosidase, is expressed at birth by severe defects in peripheral vision and hearing. The histochemical abnormalities of the peripheral visual and auditory systems in affected goats were defined in order to further investigate the expression of this "pathological region of chromosome 21 which is present in Down's syndrome (DS) after age thirty.

Patients: S-J. Richards & F. Liwii*, Dept. of Biochemistry & Molecular Genetics, St. Mary's Hospital Medical School, London W2 1PG, UK. (Spon. Genentech Laboratory, University of Helsinki, Valmonie 7, Helsinki. 

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The micrencephalic rat with its hypoplasia of the forebrain is a useful model for the study of developmental brain defects. The two most consistent changes of this rat are the misplacement and hyperactivity. Both are specific since each may arise from morphological and neurochemical alterations in several different brain regions. The markedly reduced occipital region, as well as the neuronal loss throughout the subcortical visual system, is typically associated with various developmental anomalies (Ashwell, 1987), suggesting that nodal-specific (visual) deficits should also exist. Indeed, Pereira et al. (1985) showed micrencephalic Wistar rats to be markedly impaired in visual recognition, but not brightness, discrimination. We have now replicated their results with Long-Evans rats. Mature rats learned to discriminate between two visual stimuli, vertical vs horizontal vs black and white stripes, in less trials than their normal counterparts (R=5393±266 (SEM); L=5388±280; N=8) (Abercrombie corrected). Our previous results showed that these cells secrete both PNI and PAI-1 (endothelin-like cell type PA inhibitor). We studied these inhibitors in normal brain and in micrencephalic rats. A PNI type inhibitor formed a 92 kD and 78 kD complex with 125I-proteinase inhibitor and 125I-thrombin, respectively, in the absence of SDS. In the NL tumor, the PNI complexes were increased threefold when compared to normal brain. In addition, in contrast to studies with NL in vivo, in the presence of SDS, we found another complex at 88 kD with 125I-thrombin, but no complex with 125I-proteinase. Brain tumor pathogenesis may involve increased tissue expression with protase nexin I and decreased PAI-1. Such studies are in progress. Supported by the ALS Association, NSF, Speas Foundation, and the Medical Research Service of the VA.


PNI is identical to gliarial derived nexin (GDN), a neurite outgrowth factor found in rat and human glial cells. The 9L rat brain tumor is a gliosarcoma and a model of human brain tumors. Our previous results showed that the cells secrete both PNI and PAI-1 (endothelin-like cell type PA inhibitor). We studied these inhibitors in normal brain and in 9L tumor in vivo. A PNI type inhibitor formed a 92 kD and 78 kD complex with 125I-proteinase inhibitor and 125I-thrombin, respectively, in the absence of SDS. In the 9L tumor, the PNI complexes were increased threefold when compared to normal brain. In addition, in contrast to studies with 9L in vivo, in the presence of SDS, we found another complex at 88 kD with 125I-thrombin, but no complex with 125I-proteinase. Brain tumor pathogenesis may involve increased tissue expression with protease nexin I and decreased PAI-1. Such studies are in progress. Supported by the ALS Association, Speas Foundation, and the Medical Research Service of the VA.
DEVELOPMENTAL DISORDERS

468.17 NEOCORTICAL ANOMALIES IN THE NEW ZEALAND BLACK MOUSE: A GOBLI STUDY. D. M. Ackley, S. S. E. Merchant, and A. M. Galaburda, Dyslexia Neuroanatomical Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

Some NZB mice have a unique neocortical molecular layer ectopias and laminar dysplasias (Sherman, G. F. et al., PNAS, (USA), 82: 8072, 1985). We compared over 500 cells from 3 NZB mice with 4 anomalies (NZB/w), 8 without anomalies (NZB/b), and 14 additional BXSB mice (Goabi-Stenaa). We made planimetric analyses of cell populations in the superficial portion of layer ii (iiis), the deep portion of layer ii (iid), and pyramidal neurons in layers iii, v, and vi. We quantitated basal dendritic length (bd), total number of basal dendritic branches (bdn) and of 1st (bd1), 2nd (bd2), and 3rd (bd3) order branches, number of terminal basal dendritic branches (bdh), apical dendritic length, surface area, average length per basal dendritic branch (bd/brn), and ratio of apical to basal dendritic development (ai/bai).

NZB/w had greater bdh than NZB/w in ii, greater bdh and bdh in iii and n. NZB/w had greater bdh than DBA in iii. Also, DBA showed significant interlaminar differences in most parameters, whereas only some interlaminar differences were seen in NZB/w and no differences were demonstrated in NZB/w, reflecting lesser laminar differentiation in the latter. NZB/w had a greater bdh/brn than NZB/w in ii, greater bdh and bdh in iii, and tended to have greater bdh and bdh. Ectopic neurons in i differed less from ii neurons than from iii neurons (but no difference was significant), and exhibited features of both ii and iii neurons. Qualitative differences in the ectopic neurons and implications of the findings will be discussed.

(Supported by NIH NICHD 20606 and a grant from the Carl) Herrington Foundation).

468.18 CEREBRAL AFFECTIVE CONDUCTION IN THE DEVELOPING WITCHER MOUSE AND ITS ACTING HETEROZYGOS AND NORMAL LITTERMATES. Charles B. Reynolds, Jr. and Stanley M. Berman, NINDS, NIH, Bethesda, Md.

We have transplanted heterozygous (Twl/Twl) and normals (Twl/Twl) into the brain of 12-15 day-old rat embryos, with subsequent sacrifice at 28 days of age. The animals were studied under a temperature-controlled protocol.

As previously reported (Soc. Neurosci. Abst., 11, 1985) the three genotypes clearly differed 40 days of age, when at least 90% of the animals could be identified. As might be expected the faster noces were the first to go in the (twl/twl) and iv, all of which were dead by 50 days of age. At 75 days of age the twl/iv also were not significantly different than the iv. Twl/iv animals studied at 80 days of age were significantly more than than their iv littermates.

468.19 THE RELATIONSHIP OF IMMUNE STATUS TO NEUROPATHOLOGY IN AUTOIMMUNE MICE. G. F. Sherman, FDO, Beth, G.D. Rosen, and A.M. Galaburda, Department of Psychiatry, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and Southern General Hospital, Glasgow, Scotland.

Previous studies have shown that the New Zealand Black (NZB) and BXSB mouse strains have a high prevalence of developmental cerebral anomalies (Sherman et al., PNAS, 82: 8072, 1985, and Acta Neuropathol., 74: 249, 1987). These consist of ectopic collections of neurons in the brain and are associated with underlying laminar dysplasia. The anatomical characteristics of these anomalies (dated to the late stages of neuronal migration) have lead us to propose that these mice are models for the similar anomalies seen in the brain of male dyslexics (Galaburda et al., Neurol., 18: 222, 1985). The present study was conducted to determine whether the anatomical anomalies seen in other immune-dysfunctional strains, to assess for immunological differences between strains possibly related to the presence of brain anomalies, and to evaluate intra-strain differences between mice with and without brain anomalies. At least 10 male and 10 female mice per strain were tested for these immune abnormalities. We also suspected to have immunity abnormalities (MRL/Mp or MRL/Mp/+), NZB/W, AKR, CBA, CSJ, BALB/cby-nu, N2B/6-bd/n, and additional BXSB mice to be immune in the mice and combining these strains with neonatal and subacute sera, tests for immune reactions with plasma or sera antibodies (including measurements of autoantibodies, IgG, IgA, IgM, T and B lymphocyte subsets, killer cell function, and complement activation). The brains of these mice were processed in coronal and coronal sections were cut at 35μm and stained with cresyl violet. The sections were examined under a light microscope and the incidence of anomalies was noted and the relationship to immune abnormalities analyzed.

(Supported by NIH grant 20860).

469.1 COCAINE EFFECTS ON RENIN SECRETION IN CONSCIOUS RATS. J. M. Takasugi, R. E. Balcerzak, E. A. Cunningham, K. Konigsa, and L. D. Van De Graaf, Pharmacology and The Cardiovascular Center, University of Texas Med. Branch, Galveston, TX 77550.

Our present knowledge of the neuroendocrine profile of cocaine is limited by the role of serotonin (5HT) in the regulation of renin secretion and the marked effects of cocaine on spontaneous activity of 5-HT neurons recorded in the nucleus raphe nucleus. We investigated the effects of systemic cocaine exposure on plasma renin activity (PRA) and concentration (PRC).

Male Sprague-Dawley rats received cocaine (15mg/kg, 1p.). or saline 15 min prior to decapitation. Trunk blood was collected, plasma separated, stored at -80°C, and assayed for PRA and PRC as previously described. We found an acute increase in PRA and PRC of 50% dose of cocaine (COC) significantly (p<0.01) decreased PRA and PRC as compared to saline (saline: 1.5±0.2 vs COC 9.5±1.2 vs SAL 16.2±0.7, respectively). Examination of the time course of this cocaine effect revealed a maximal suppression of renin secretion at 15 min in a dose-dependent manner. PRC was also evaluated in animals sacrificed 24 hr following repeated exposure to cocaine (15mg/kg, 2x daily, 1p. for 7 days) or saline. Animals treated with cocaine had elevated PRC levels as compared to saline controls (COC 9.8±1.0 vs SAL 6.1±0.6) without changes in plasma corticosterone. These observed changes in renin secretion may result from potent effects of cocaine on 5-HT neuronal systems. Supported by DA 04296.

469.2 SEROTONIN-INDUCED NATRIURENIS IS MEDIATED BY RENAL NERVES AND ARF IN THE UNANESTHETIZED RAT. R. Moyes*, A. R. Johnson (SPOR: I. Gormadano), Dept. of Psychology and Pharmacology and The Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242.

Central serotonin (5-HT) has been proposed to be involved in the control of sodium excretion. We studied the role of renal neural natriuretic peptide (RNP) and angiotensin I (ANGIOT) on natriuresis induced by intravenously-administered (IVT) 5-HT. Animals were bilaterally renal or sham denervated and implanted with ventral, comitant, renal nephron. One week later, they were instrumented with renal venous and arterial catheters and a bladder cannula. The next day, rats were exposed to a hypertonic solution, and mean arterial pressure, sodium, and angiotensin I were measured as the ivt 5-HT excretion were determined. After 150 min, 5-HT (20 μg free base) or vehicle was injected IVT. In a second experiment, plasma ANP levels were measured after 60 min after 5-HT (30, 20 μg). Intravenous 5-HT produced a decrease in sodium resorption, consistent with a withdrawal of sympathetic tone. These findings also significantly attenuated the natriuretic response to 5-HT. Both doses of 5-HT caused a significant elevation of plasma ANP levels. These results suggest that the natriuretic effect produced by centrally administered 5-HT may involve release of renin as well as ARF release.

Supported by Research Grants NHI 59911338 and Iowa Heart Association 87-G-15.
NEUROENDOCRINE CONTROLS: OTHER III


Our previous studies employing microdialysis in urethane-anaesthetised female rats demonstrated that ovarian steroids may modulate norepinephrine (NE) release in the ventromedial hypothalamus (VMH). The present experiments measured NE release in the VMH of awake rats that were ovariectomized and steroid-treated. One day after rats were treated with either oestradiol benzoate (EB), and two additional samples were collected to monitor possible stress effects on NE release. Twenty-four hr after EB injection, sample collection resumed for 4-6 hr; some animals received KCl stimulation in the dialysate to induce depolarization-evoked release of monoamines. During sample collection on the following day (48 hr after EB), rats were injected with 200 µg of progesterone (P) and were tested for estrous behavior with stimulus males 3.5 hr later. Preliminary results show that animals chronically implanted with dialysis probes in the VMH exhibit robust, hormone-dependent estrous behavior. We are able to detect NE in VMH dialysates of freely moving female rats, and we will present data correlating NE release with EB and P activation of estrous behavior.

469.5 CIRCUANNUAL VARIATIONS IN PINEALOCYTE SYNAPTIC RIBBONS IN THE L-3-LINED GROUNDSQUIRREL. J.A. McNULTY*, W.A. SPURRIER* (SPON: F. Lavelle). Deps. of Anatomy and Neurosurgery, Loyola Univ. Stritch School of Medicine, Maywood, IL 60153.

The synaptic ribbons (SR) in a pinealocyte organelle that is believed to play a role in the photoperiodic sensitivity of melatonin. In this study, SR numbers were quantified at monthly intervals over a yearly cycle (June-July) in the hibernating and seasonally reproductive ground squirrel, Citellus tridecemlineatus. Glands (4/month) of both sexes were collected at the midpoint of the photoperiod, processed for routine TEM and SR counted in 3 grid spaces (300 mesh) from 1 section per gland. SR numbers were high in active animals from May to Oct (35±45/10,000 µm²). In the months of Nov and Dec when squirrels were hibernating, SR frequency markedly declined (6±7 fold). There was a gradual increase in SR numbers during the period of late hibernation (Jan, Feb) and sexual maturation (Mar-May). Numerical changes in SR were due to changes in both ribbons and spherules, as well as in the proportion of which occurred in pairs. Although ribbon fields of up to 9 SR were observed, there were no consistent seasonal differences in fields comprising 3 or more SR. The winter decline in SR frequency is consistent with reports of a decrease in pineal melatonin during hibernation and supports the hypothesis that pinealocyte SR are an important role in neurotransduction of melatonin biosynthesis.


Gap junctions (GJs) mediate electronic and metabolic coupling. Extensive morphological studies have shown gap junctions between pinealocytes in diverse species. Here we characterize their physiological, biochemical properties and GJ permeabilities. The present experiments indicate that GJs are present between pinealocytes isolated from adult male and female Sprague-Dawley rats (ca. 200g) were used. Anesthetised rats were decapitated, and the pineal gland was dissected out and placed in Dulbecco's saline. After decapsulation, 2-3 glands were minced and incubated with 0.5% trypsin and 0.06% collagenase (17 U/mg) in nutrient medium (RPMI/F12, 1:1 Gibco) for 1 h at 37°C. The tissue was triturated in RPMI/F12 with 10% FCS, and dissociated cells were day 2 polyethylene coated coverslips. After an hour, electrical coupling was evaluated in cell pairs under double current clamp. Dye coupling was evaluated by injecting Lucifer yellow into one cell of a pair or a cluster. The incidence of electrical coupling was 35%. Mean junctional conductance was 113.4 nS, range 2-400 nS, n=75. Dye coupling was seen in 30% of injected pairs (n=28) and in multiple cells of clusters. Octanol (0.4mM) rapidly and reversibly uncoupled the cells. Physiological saline also uncoupled reversibly, but more slowly consistent with removal of a nutrient factor. No immuno-labeling was seen with antibodies to the rat liver 27 kDa GJ protein, but immunoreactivity was found with an antibody specific for mouse liver 21 kDa protein. The labelling showed a spider-pattern, which is consistent with a burst of freeze fracture micrographs of pineal GJ. Modulation of GJs between pineal cells may be important in controlling secretion and synchronizing their circadian rhythm.

469.7 EPILEPTIC CONVULSIONS IN PINEALECTOMIZED MALE GERBILS. T.N. CHAMPNEY. Deps. Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Pinealectomy (PX) of male gerbils produces grand mal seizures and decreases in cortical norepinephrine (NE) levels within 45 minutes of the surgery. The present studies determined the role of the pineal stalk in pineal mediated convulsions, measured catecholamine levels in numerous brain regions and quantified the role of PX on the sensitivity of gerbils to pentylenetetrazole (PTZ)-induced seizures. Nine week old male gerbils were PX, sham-PX or had their pinealomas anesthetized. After arousal from anesthesia, stalk transection produced convulsive activity and depressions (p<0.01) in cortical NE levels (in vivo surgery) to the same degree as observed in PX gerbils when both were compared to sham-PX. Depressions in NE were only observed in the perialretial cortex observed in the hypothalamus, amygdala or hypothalamus. Gerbils were also PX and sham-PX under pentobarbital anesthesia (which prevents convulsive activity for two weeks) and injected with one of 4 doses of PTZ (15, 30, 45 or 60 mg/kg). PTZ produced increased convulsive activity with increased dosage, and was equally effective in PX, sham-PX or control gerbils. Therefore, PX does not alter the gerbils' responsiveness to another convulsant agent, PX. Also, PX-induced convulsions appear to be due to an intraneuronal thalamic discharge within the pineal stalk with a concomitant decline in cortical NE levels.
649.9  
EFFECTS OF MELATONIN AND IMIPRINE ON BRAIN SEROTONIN AND 5-HIAA LEVELS OF MALE SYRIAN HAMSTERS.  N. M. McCashin, J. Vriend* and J. Vriend* (SPON: G. K. W. Cheng). Department of Anatomy, Univ. of Manitoba, Winnipeg, MB, Canada R3E 0W3.

In this study the effects of melatonin and imipramine administration on serotonin and 5-HIAA levels were determined by HPLC-EC in selected brain regions of the Syrian hamster. Melatonin (20 mg/kg), imipramine (0.75 mg ip), both melatonin and imipramine, or saline were administered daily for 8 weeks to male hamsters assigned to groups of 12. At the end of 8 weeks the animals were killed. Testicular weights were recorded at this time.

Four animals of each group were treated with pargyline (20 mg/kg) 24 hours prior to sacrifice. Serotonin levels were significantly depressed by melatonin in brain stem and in hypothalamus. Imipramine administration significantly increased 5-HIAA levels of brain stem. A small decrease in serotonin content (p < .01) after pargyline administration was observed only with melatonin. Melatonin administration also resulted in significant increases in hypothalamic content of the dopamine metabolites, DOPAC and HVA. Imipramine administration did not prevent the melatonin induced gonadal involution or the melatonin induced inhibition of gonadotropins. The effects of imipramine on the serotonergic system are interpreted as increased metabolism by MAO concurrent with inhibition of the reuptake mechanism.

649.11  

Zinc (Zn) is an essential trace element in man. The requirement for Zn stems partly from its role in a variety of enzymes including those required for DNA and RNA synthesis. In the central nervous system (CNS) Zn is sequestered in specific regions, e.g., the mossy fiber pathway of the hippocampus where it appears to be associated with neurotransmission. In addition, intracerebral endocrine tissue has a higher concentration of Zn than the brain as a whole, and recent studies indicate that Zn is a regulator of hormone secretion. At physiological concentrations Zn suppresses the output of prolactin from dispersed pituitary cells in vitro (Judd et al., Brain Res., 294: 190, 1984). Further study of the role of Zn in endocrine-CNS interaction is warranted. Identifying regions rich in Zn is a first step to that end.

In this study brain and endocrine tissues were obtained from 7 male rhesus monkeys and freeze-dried at -80°C. Zinc concentration was determined by flame atomic absorption spectrophotometry. Concentrations in ug Zn/g dry tissue are: Temporal lobe 70 ± 4, Neurohypophysis 84 ± 30, Adenohypophysis 137 ± 59 and Pineal 162 ± 80. Endocrine tissues were found to have significantly higher concentrations than the temporal lobe, and in addition, the adenohypophysis had a significantly higher concentration than the neurohypophysis.

649.13  
EVIDENCE FOR DENDRITIC AND AXONAL HORMONE RELEASE IN THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM.  C.D. Twohey, E. S. Ghiggia & G.I. Hatton. Neuroscience Program, Dept. of Anatomy & Physiology, College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

In order to observe hormonoreleasing sites in the rat neurohypophysis the tannic acid-Ringer technique of Buma and Nieuwenhuyzen (Neurosci. Lett. 74: 151, 1987) was utilized. Three rats with normal levels of k+ in the perfusion mixture showed only rare exocytotic figures in the neurohypophysis (10-20% of testis). In contrast, the density of PCP receptors in target endocrine organs provides support for peripheral sites of action.

Upon examination of the supraoptic nuclei from the same animals it was found that in the rats with high K+ occasional exocytotic figures of dense core granules occurred in dendrites, but not in somata. This apparent dendritic release could accomplish local information transfer and/or be a source of the oxytocin and vasopressin found in the CSF. Current experiments are underway to see if similar findings are obtained with more physiological stimulation of hormone release. Supported by NIH grant NS09140 and by a MSTP fellowship to GKS.

649.10  
EFFECTS OF MELATONIN AND IMIPRINE ON CIRCULATING LEVELS OF PROLACTIN AND THYROXINE IN PARGYLINE TREATED HAMSTERS.  J. Vriend* and M. R. McCash* (Spon: J. A. Paterson). Department of Anatomy, Univ. of Manitoba, Winnipeg, MB, Canada R3E 0W3.

The present study was designed to determine the effects of imipramine and pargyline on the melatonin-induced circulating levels of prolactin (PRL) and of the melatonin-induced inhibition of circulating levels of thyroxine (T4). Melatonin was administered daily (25 ug sc for 8 weeks) to male Syrian hamsters (N=12) alone, or in combination with imipramine (5 mg/kg ip). Additional groups of hamsters were treated with imipramine alone or with saline alone. All injections were administered 1-2 hours before lights out in an animal room maintained on a 14:10 hour schedule. At the end of 8 weeks the animals were killed. Four animals of each group were treated with pargyline (20 mg/kg) four hours prior to sacrifice. Serum was collected for assay of T4 and PRL by RIA. As in previous studies melatonin significantly reduced circulating levels of these hormones. Four hours after pargyline a 3-fold increase in PRL was observed in imipramine treated hamsters, but not in saline or melatonin injected hamsters, nor in hamsters treated with both imipramine and melatonin. Pargyline also resulted in an increase in T4 in imipramine treated hamsters, but not in hamsters treated with Imipramine and melatonin.

649.12  

PCP and the prototypic sigma agonist N-allylnormetazocine (NAMN) have been reported to alter neuroendocrine functions (stimulation of ACTH and corticosterone release and suppression of prolactin and luteinizing hormone secretion in rats). The aim of the present study was to survey endocrine organs and to identify, characterize and localize a and PCP receptors, if present, in pituitary, adrenal, testis, ovary and placenta. Sigma receptors were labeled with [3H]-haloperidol in the presence of 25 nM imipramine or with 1.3·10-6 M [3H]-dihydro-5-HT3 receptors in hypothalamus and pituitary, ovary and placenta. Picrotoxin (300 µg) and strychnine (5 µg) were included in the solution. Sigma receptors were localized by autoradiography and endocrine manipulations (e.g., hypophysectomy) are currently being used to localize and identify the cells containing PCP and σ receptors.

While the sites of action of PCP and NAMN in altering hormone secretion remain to be determined, our demonstration of high densities of PCP and σ receptors in target endocrine organs provides support for peripheral sites of action of these drugs.

649.14  

Isoproterenol alters the morphology of pituitary gland of the rat. The neurointermediate lobes from adult male rats were isolated and placed in chambers containing artificial CSF at 37°C. Treatment with isoproterenol caused a change from flattened to stellate morphology in cultured adult pituitaries. We sought to determine if similar changes could also be observed in the isolated neural lobe. Neuronal intermediate lobes from adult male rats were isolated and placed in chambers containing artificial CSF at 37°C. Treatment consisted of a 15 min. incubation with CSF containing either 0.2% aspirate or isoproterenol. Daily 0.2% aspirate. Tissue was then prepared for conventional electron microscopy after which morphometric evaluation of the percent of, pituitary and neurointermediate, membrane contact with the basal lamina was performed. Isoproterenol stimulation resulted in a significant decrease in pituitary and increase in neural contact with the basal lamina, as compared to control. These changes are similar to that observed during conditions which require increased hormone release (e.g., dehydration or lactating). These results suggest that β-agonist mediated changes in pituitary morphology may play a major role in the mechanism underlying these phenomena. Supported by NIH grant NS09140 and by a MSTP fellowship to GKS.
469.15
INHIBITORY EFFECT OF NOREPINEPHRINE (NE) ON THE ELECTRICAL ACTIVITY OF CAUDBLUE-PROJECTING PARAVENTRICULAR (PVN) NEURONS IN RAT. Y. Kikus, C.A. Dudley and R.L. Moss. Dept. of Physiology, Univ. of Texas Southwestern Medical Center, Dallas, Texas 75235.

Utilizing conventional extracellular single unit recording techniques, the effect of iostrophopherically applied NE on the activity of caudally-projecting PVN neurons was assessed in male Sprague-Dawley rats anesthetized with urethane. These PVN neurons were identified antidromically by electrical stimulation (50 µA - 1.5 mA) of the caudal ventrolateral medulla (mean ± SEM antidromic latency: 34± 2.4 ms). In 15 of 19 identified PVN neurons (mean ± SEM baseline firing rate: 2.7± 0.6 impulses/s), NE was demonstrated to be inhibitory. No excitatory NE effect was observed. Five of those 15 neurons were also antidromically identified as projecting to the posterior pituitary (mean ± SEM antidromic latency: 15.0± 2.5 ms). The inhibitory action of NE was selectively blocked by the alpha antagonist, phentolamine (α7) that was co-iontophoresed with NE, but not by the beta antagonist, timolol (α9). In contrast to the inhibitory action of NE on the caudally-projecting PVN neurons, an excitatory action of NE was frequently observed in the PVN neurons projecting only to the posterior pituitary. Supported by HD09988-V.

469.16
PARABRACHIAL NUCLEUS INVOLVEMENT IN THE INHIBITION OF DEXAMETHASONE-INDUCED VASOPRESSIN RELEASE BEYOND WATER INTAKE. L.E. Ohman, E.E. Shade and J.R. Haywood. Dept. of Pharm., The Univ. of TX Hlth. Sch., San Antonio, TX.

High circulating levels of vasopressin (pAVP) induced by water deprivation (WD) decrease within minutes of water ingestion. The lateral parabrachial nucleus (LPBN), known to be involved in the regulation of water intake and AVP release, may contribute to this inhibition of WD-induced pAVP. Rats were given either bilateral electrolytic or sham tract lesions of the LPBN. Water was removed for 48 hr, after which the rats were allowed to drink for 1 hr. Plasma osmolality (Osm) and AVP were determined prior to WD, before access to water; and 10 min after the onset of drinking. Plasma Osm increased from 289±2 to 298±2 mOsm in lesioned rats and from 288±2 to 296±2 mOsm in sham-lesioned rats in response to 48 hr WD. Plasma AVP levels also increased in both groups (Lesion: 1.0±3.1 to 13.5±2.4 µU/ml; Sham: 0.7±10.2 to 15.4±3.4 µU/ml). Lesioned rats drank significantly more water (18±61.5 ml) than sham rats (11±5.06 ml), but pOsm decreased to a similar value in both groups (Lesion: 284±5 mOsm; Sham: 291±2 mOsm). Plasma AVP decreased 10.6±1.4 µU/ml (75%) in sham lesioned rats after water intake, while the pAVP decrease in lesioned rats, 5.7±1.5 µU/ml (45%), was significantly less. These data suggest that the LPBN partially mediates the inhibition of dehydration-induced pAVP release by water intake. (Supported by HL39277.)

469.17

Glucagon secretion in response to insulin hypoglycemia is impaired in diabetic patients and rats and this impairment correlates with diabetic neuropathy. We have recently shown that glucagon is secreted in response to direct vagal nerve stimulation and that this response is also impaired in diabetic rats (FASEB J. 2: A1594, 1988). The present study expands on the vago-glucagon secretion in galactosemia and diabetes. Rats were anesthetized with chloral hydrate (350 mg/kg i.p.) and the carotid artery was cannulated for blood sampling. Vagal nerves were stimulated via silver electrodes for varying times at fixed frequency (20 Hz). Hypoglycemia-induced glucagon secretion was observed by constant infusion of insulin, via the jugular vein, until plasma glucose levels dropped to 40 mg/dl. Plasma glucose and glucagon levels were determined by glucose oxidase and RIA respectively. Plasma glucagon levels increased with increasing duration of vagal stimulation. This vagal-stimulated, as well as hypoglycemia-induced, secretion was totally prevented by atropine pretreatment (2 µg/kg i.v.). Furthermore, carbaryl infusion resulted in elevated plasma glucagon. Glucagon secretion was either left or right vagal stimulation. Although insulin-induced glucagon secretion prevented by atropine it was not eliminated by acute bilateral vagotomy. In addition to diabetes, galacose-fed rats exhibited a similar impairment of glucagon secretion. It is concluded that glucagon secretion is regulated, in part, by cholinergic influences that may involve the vagus nerve, but may also involve other systems. (Supported by U. Cincinnati Research Council.)

469.18

Receptors and immunoreactive perikarya for angiotensin II (AII) are populated densely in neurally-linked subunits of the medullary dorsal vagal complex (DVC) - the area postrema (AP), nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus (DMN). We hypothesized that metabolic activity in DVC would be stimulated by dehydration associated with high systemic AII. We applied the [14C]deoxyglucose method in conscious Sprague-Dawley (SD) rats, SD rats deprived of water (WD) for 18 hr. Values for glucose metabolism (GM) in control SD rats were AP (0.9±0.3 µmol/g/min), NTS (1.5±0.4 µmol/g/min), and DMN (1.6±0.4 µmol/g/min). Similar values were found in LE rats. GM in WD rats increased GM of AP, NTS, and DMN by 38%, 35% and 21%, respectively. In DI and WD-DI rats, GM increased by 12 to 15% in AP and NTS; no change occurred in DMN. WD-DI rats had higher GM in AP and NTS than water-deprived DI rats. We speculate that AII-enriched regions within DVC are activated by circulating AII via the AP in these rat models of dehydration.
HYPOTHALAMUS I

THURSDAY PM


The results of the present study indicate that the hypothalamus modulates jaw closure, but little is known of the interaction between peripheral receptors and hypothalamus in regulating this response. We observed peripheral (perioral) stimulation of isolated periodontal ligament upon the magnitude of maesthetic EMG activity in the presence of hypothalamic stimulation, which was elicited by hypothalamic stimulation alone.


Neurons with low-threshold calcium spikes (LTS) have been found immediately lateral to the paraventricular nuclei (Poulain, P. and Carette, B., Brain Res. Bull. 19:465-1987). These neurons are distinguishable from non-LTS neurons in the nucleus, not only by the presence of LTS potentials, but also by their afterhyperpolarizations, current-voltage relations and baseline synaptic activity (Tasker, J. G. and Dudek, F. E. Soc. Neurosci. Abstr. 13:1370, 1987). For the present study, three pools of rats (male, 600-800 g) were pressure-applied to several locations around the basis of the hypothalamus (PVN) and the dorsomedial hypothalamus (DMH). The animals were anesthetized with sodium pentobarbital (60 mg/kg IP) and the animals were killed by decapitation. The brains were removed and immersion-fixed in 4% paraformaldehyde. The brains were sectioned in 50-µm-thick sections and placed in a solution of 5% sucrose. Following cryoprotection, the brains were sectioned in 50-µm sections. A total of 10 sections were collected from each animal. The sections were then processed with a standard immunohistochemical protocol using a rabbit anti-ATPase antibody. The sections were then incubated with a secondary antibody conjugated to biotin. The sections were then incubated with avidin-biotin complex and finally incubated with diaminobenzidine. The sections were then counterstained with neutral red. The sections were then examined under a light microscope. The results of the present study indicate that the hypothalamus modulates jaw closure, but little is known of the interaction between peripheral receptors and hypothalamus in regulating this response. The results of the present study indicate that the hypothalamus modulates jaw closure, but little is known of the interaction between peripheral receptors and hypothalamus in regulating this response. The results of the present study indicate that the hypothalamus modulates jaw closure, but little is known of the interaction between peripheral receptors and hypothalamus in regulating this response.

The OVLT, a circumventricular structure involved in hydromineral homeostasis, contains cells traditionally linked to the nuclei mediobasal hypothalami (NM), supraoptic (SON) and paraventricular (PVN) nuclei. However, there is scant information on the electrical properties. Intracellular recordings obtained from 42 OVLT neurons in slices and superfused explants of rat brainstem revealed action potential resting membrane potentials near -65 mV, input impedances of 80-280 MΩ, linear V-I plots below resting membrane potentials and low threshold calcium spikes. Antidromic activation from the region of NM, PVN and/or SON agreed with axon branching and their trajectories as visualized in Lucifer yellow filled cells. The latter also revealed a simple cellular morphology (soma 10-15 μm, 0 dendrites). Each of 17 tested cells depolarized in hyperosmotic (+16 mosmol) media. These data suggest an intimate relationship of OVLT neurons with forebrain areas regulating body fluid balance. Supported by FCAR, QMA and MRC.

470.9 CHARACTERISTICS OF THERMOSENSITIVE AND OSMOSSENSITIVE NEURONS IN THE RAS DICEPHALON. R.A. Travis* and J.A. Boulaert, Department of Physiology, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Hypothalamic neurons respond to various homeostatic challenges, including changes in temperature and osmolarity. The study evaluated diencephalic neuronal thermosensitivity and osmosensitivity in Sprague Dawley, Wistar-Kyoto, and spontaneously hypertensive rats. In vitro neuronal activity was recorded from several nuclei in horizontal tissue slices perfused with control (300 mOsm/kg) or hyperosmotic (280 mOsm/kg) and hypotonic (320 mOsm/kg) cerebrospinal fluid (ACSF). The majority of neurons responding to at least one osmotic stimulus showed no specific pattern of response to the test media. Temperature insensitive neurons to changes in temperature showed no change in the hypothalamic medium, and either increased or decreased their spontaneous activity.

While there were no differences in the responses of hypertensive and normotensive rats, there were differences in the responses of temperature sensitive and insensitive neurons to changes in osmolarity. (Supported in part by NSF, AHA and NIH grants.)

470.11 PERFUSATION P02 AND NEURONAL THERMOSENSITIVITY IN PREFRONTAL AREA (POA) SLICES. M. Shibata and C.M. Blattei, University of Tennessee, Memphis, TN 38163.

In electrophysiological studies, it is usual to gas the perfusates of brain slices with 95% O2/5% CO2 whereas 21% O2/5% CO2 is normally used to maintain brain tissue cultures. To determine whether 95% O2 might be needlessly high, passive afferent neuronal excitability, we compared the effects of the gases on the firing rates (FR), spontaneous firing rates in 300-μm-thick slices from guinea pig parafascicular thalamus perfused at 1 ml/min with artificial cerebrospinal fluid (ACSF) gassed with 95% O2 or 21% O2/5% CO2. Thermosensitivity (Q10) was assessed by FR changes with passive afferent nerve stimulation (20 Hz, 2 μA). Temperatures were controlled with a water jacket (25 or 37°C), according to established criteria. Results: 1) Within 1.5 h, incubation chamber perfusate PO2 at 37°C reached 300-350 mmHg and remained constant gassed with 21% O2 and 150-160 mmHg with 21% O2. 2) Of 7 thermosensitive units initially characterized in 21% O2/ACSF, 4 units lost their thermosensitivity and increased their Q10, 4 units increased spontaneous firing when 95% O2/ACSF was substituted. 4 units lost their Q10, 4 units increased spontaneous firing when 95% O2/ACSF was substituted. 3) However, the Q10 values measured in 21% O2 were higher than in 95% O2: These findings suggest that exposing to 21% O2 decreases PO2 to an unphysiological range and may cause impairment of POA neuronal responses to thermal stimuli.


Alpha-melacoycte stimulating hormone (α-MSH) has been implicated as an endogenous antipyretic active during the rising phase of fever. Since brain α-MSH content is increased in the arcuate region, we hypothesized that lesioning of this area, with monosodium glutamate (MSG), would reduce the levels of α-MSH and alter the fever responses induced by central injections of prostaglandin (PGE1). Wistar pups were given randomly either ip injections of €80% (4 ng/kg body wt) or equal volume of saline on day 1, followed by saline on day 2 and 4 after birth. At maturity, male rats were implanted with intraventricular (ICV) cannulas and abdominal temperature transmitters. After recovery from surgery, the animals were given ICV injections of PGE1, (20 ng) and body temperature data were computer-collected every 5 min for 2 h before and 4 h following injections. After completion of the experiment, brain tissue was assayed for α-MSH. The results show that the fever in the MSH treated animals rose faster and was higher (p<0.05) than fever in the saline group during the first 20 min. As MSH posttreatment decreases α-MSH levels in brain, the enhanced fever in the MSH treated animals supports the hypothesis that α-MSH is an endogenous antipyretic. (Supported by Mt.St.Vincent and the MRC.)


Responses to noxious and sexual stimuli have visceral components which are modulated by opiates. Although the VMH is known to be involved in the control of both pain and male sexual behaviour, the role of the PVN has not been tested despite its role in analgesia and male sexual behaviour. The role of the VMH in the control of pain was examined in the role of the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN) in the dual control of analgesia and sexual behavior. Bilateral lesions were made in the VMH or PVN of male rats with betocaine and the effects of saline, naloxone (4 mg/kg) and morphine (10 mg/kg) on the analgesic threshold to heat (tail-flick test) and pressure (pressure algometer) were tested. Drug treatments were repeated and the effects on male sexual behavior tested. There was a significant and modality-specific effect of the lesions on analgesia. The VMH group was hyperalgesic to heat, the VMH group was hyposensitive to heat and the VMH group was hypo-sensitive to both drugs. There was a modest reduction in mounting behavior following naloxone treatment, whereas naloxone had no effect on the VMH and control rats. Thus the lesions in the VMH and PVN have opposite effects on both male sexual behavior and pain and the effects on analgesia are modality-specific. The drug effects indicate that the lesions produce an antagonism in opioid systems, and these alterations in turn exert opposite effects on both sexual behavior and pain. The results after naloxone lesions further suggest that the AHS may play a role in the modulation of male sexual behavior and the response to pain. Supported by M.R.C.

In the adult male rat brain aromatase activity is regulated by androgens. We have investigated whether developmentally regulated (DHT) and/or pituitary aromatase activity is regulated by androgens. Two- and 8-week-old male rats were divided into three groups: intact controls, castrates for 2 weeks, and castrates treated with 20 mg DHT/kg body wt. At 28 days (28-juvenile), 68- (pup-puberal) and 68- (post-puberal) days of age the hypothalamic and pituitary were incubated with [1α, 1β-3H]testosterone (T). Aromatase activity was estimated by measuring the release of [18-H] from T into water. The average activity decreased markedly in the pituitary and hypothalamus of postpuberal rats. Castration increased the activity in the pituitary at all ages tested and DHT treatment suppressed the increase. The response of hypothalamic aromatase to castration and castration plus DHT replacement was opposite to that of the pituitary. The results indicate that aromatase activity is regulated differently by androgens in the hypothalamus and pituitary during sexual development and suggest that the post-puberal decrease in pituitary enzyme activity can be attributed to androgens while the decline in hypothalamic enzyme activity is androgen-independent.


The tuberal, or retrochiasmatic, portion of the supraoptic nucleus (SON) consists largely of vasopressin neurons whose dendritic and axonal morphology have not been described. SON neurons impaired in hypothalamic hypophysiotropic explants in vitro were filled with biocytin (Horikawa and Armstrong, J. Neurosci. Meth., in press) and were occasionally observed, emitting from both somata and dendrites. The beaded axon arose from either the soma or a primary dendrite. Hair-like appendages arising from axonal dilatations were fairly common. SON neurons possessed 2-4 sparsely branching, varicose dendrites which were largely oriented in the horizontal plane. Spines were occasionally observed, emitting from both somata and dendrites. The headed axon arose from either the soma or a primary dendrite, and coursed in a wide dorsomedial arc before turning caudally into the neurohypophyseal tract. In some cases axons were traced as far as 2 mm from the soma, almost reaching the neural stalk. Short, hair-like expansions arising from axonal dilations were fairly common, but long collaterals were not observed. SON neurons displayed a prominent afterhyperpolarization and firing frequency adaptation which distinguished them from a more dorsal group of neurons which fired rapidly and displayed low-threshold, slow action potentials, but which could not be stimulated antidromically. Supported by NIH grant NS29341 (WEA).


NFG induces in PGC cells a 50 enhancement of mRNA coded for the VGF gene (Science, 229:393). Two different restriction fragments from a VGF cDNA clone were fused into the beta-galactosidase (B-gal) backbone. Antisera made in rabbits against each of the two fusion proteins stained neurons in the rat suprachiasmatic nucleus (SCN). In addition to diffuse, cytoplasmic staining, intrinsic and projection axons of the SCN were labeled. The expression of the VGF related protein was found in the dorsomedial area of the SCN where cells also contain vasopressin, and in SCN of Brattleboro rats lacking the vasopressin gene. Perikarya, but not axons, of the magnocellular paraventricular and supraoptic nucleus showed some weaker positive immunostaining. A low level of immunoreactivity was also seen in some other hypothalamic brain areas. SCN immunostaining was not blocked by bacterial B-gal lyase absorption; different antisera made against the product of an unrelated cDNA fusion to B-gal showed no SCN immunostaining. Adult SCN expressed no immunoreactivity for NFG-receptor, while control cells of the medial septum did. These results indicate that a previously unidentified protein coded by the VGF gene is strongly expressed in a subpopulation of SCN cells and axons, and that this protein may not require NFG for a strong expression in the CRB.


Two classes of voltage-sensitive fluorescent dyes are known which respond to changes in the cell membrane potential by varying their intensity of emission. In the case of the cation-sensitive DIOC(6) (3), cell depolarization produces a drop in fluorescent output. We have used this dye to examine stimulus-induced depolarization in fetal hypothalamic neurons, obtained from Neurons were dissociated by enzymatic treatment followed by mechanical dispersion and the cells loaded with 20 nM DIOC(6) (3) at a concentration of 10^6 cells/ml. The addition of NGF and corticosterone (50-100 μM) depolarized the majority of these cells. Analysis of fluorescence with time revealed that a large proportion of cells begin to repolarize after 5-6 minutes, bringing their mean resting membrane potential close to pre-stimulation levels. The glutamate analog, NMDA (10 μM), also depolarized 40-50% of cells. An interesting corollary to these experiments is the K^+ induced cell swelling, which does not appear to be attributable to chloride concentration changes in comaturity. Parallel studies of stimulus-induced calcium mobilization with these cells will be presented in another presentation (Grierson et al). Supported by NIH NS25168 and a grant from the UMDNJ Foundation.

The somas of primary afferent neurons in the mesencephalic nucleus of the trigeminal nerve (Mes V) in rat and mouse are surrounded by a dense plexus of adenosine deaminase-immunoreactive (ADA-ir) axons which originate, in part, from ADA-ir neurons located in the tectofugal sector of the lateral geniculate (LG) and pulvinar (Pulv) nuclei. Immunohistochemical studies suggest that the distribution pattern of TH-ir neurons in cats and monkeys may be governed by the gonadal steroids in both an organizational and activational manner, is present in the periventricular hypothalamic (POA) nuclei of the rat hypothalamus, and can be modulated by the anterior pituitary gland. The physiological significance of this distribution pattern remains to be determined.

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HIS TOCHEM IS FY FOR NEUROPEPTIDE Y AND PHENYLETHANOLAMINE N-M ET HYL T RAN S-LEYLANDYN T R A S- ACETYLIC ACID TRANSAMINASE (PAT) IN THE POSTERIOR HYPOPHYSIS. A. A. C. Castren, S. J. S. Wiegand, and G. E. P. Commody, Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536, Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

The posterior hypothalamic nucleus (POA) is a major site of neuroendocrine regulation in many species. The POA is a complex region that contains several neuronal populations that are involved in the regulation of reproducitve function. The POA has been shown to contain a variety of neurotransmitters and neuropeptides, including oxytocin (OXY), vasopressin (VP), and neuropeptide Y (NPY). These neuropeptides are involved in the regulation of a variety of physiological processes, including reproductive function.

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POTENTIAL TARGET NEURONS OF A SEXUALLY DIMORPHIC ENKEPHALINERGIC NEURON IN THE POSTERIOR HYPOPHYSIS. A. A. A. Watson, Jr., M. C. M. Langub, Jr.*., S. J. S. Wiegand, and G. E. P. Commody, Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536, Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642, and Dept. of Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

A sexually dimorphic enkephalinergic fiber system has been described in the posterior hypothalamic nucleus (POA) of several species. This system is characterized by the expression of a specific peptide, enkephalin, which is stored in neurosecretory granules. The expression of this peptide is regulated by gonadal steroids, and the neural circuitry of this system is thought to be involved in the regulation of reproductive function.


Localization of tyrosine hydroxylase immunoreactive (TH-ir) neurons in the posterior hypothalamus of cats and Rhesus monkeys was studied in detail. Our study of the distribution pattern of TH-ir neurons in cats and monkeys with in vivo retrograde-horseradish peroxidase method showed numerous TH-ir neurons in the tectofugal sector of the lateral geniculate and in the pulvinar. In the periventricular nucleus and the posterior hypothalamic nucleus of both species, interneurons containing TH-ir neurons were present. The distribution patterns of these TH-ir neurons in cats and monkeys were similar to those described in other species, and neurons were also present in other regions of the hypothalamus, including the dorsomedial hypothalamic nuclei. These areas were not recognized in earlier studies to contain catecholaminergic neurons. The distribution patterns of TH-ir cells in hypothalamus of cats and monkeys show many similarities to the patterns described in mice using immunohistochemical methods. In addition to these regions, other TH-ir neurons were also noted prominently in the preoptic, suprachiasmatic, and the dorsomedial hypothalamic areas. These areas were not recognized in earlier studies to contain catecholaminergic neurons. The distribution patterns of TH-ir cells in hypothalamus of cats and monkeys show many similarities to the patterns described in mice using immunohistochemical methods. In addition to these regions, other TH-ir neurons were also noted prominently in the preoptic, suprachiasmatic, and the dorsomedial hypothalamic areas. These areas were not recognized in earlier studies to contain catecholaminergic neurons.

Among the long-lasting cardiovascular regulatory control network is an inhibition of phrenic nerve output, evoked by brief exposure to hypoxia, which lasts at least one hour and requires catecholamines, bradykinin, and prostaglandins. It is likely that hypoxia facilitates release of a humoral factor from the brainstem which is involved in the long-lasting cardiovascular response to hypoxia. The aim of the present study was to investigate the neurochemical basis for this inhibition. All studies were performed in cats anesthetized, artificially ventilated, and with a constant arterial CO2 level of 40 mmHg by a Harvard respirator. Cervical vagal nerve activity was monitored using bipolar electrodes. Axonal activity of the phrenic nerve was then monitored using extracellular recording techniques. The phrenic nerve drive was determined as the product of peak integrated phrenic nerve activity and phrenic rate. Cycles were then analyzed, and the phrenic nerve activity and phrenic rate were determined. The results suggest that the long-lasting inhibition of phrenic activity is mediated by a humoral factor which is released by the brainstem in response to hypoxia.
In some experiments both glutamatergic and electrical LC activation were used to identify contributions from different brainstem nuclei. Electrical LC activation can produce reliable and significant enhancement of the perforant path (PP) evoked population spike when administered to the norepinephrine (NE) nucleus. However, only certain nuclei can produce long-lasting enhancement of the PP evoked population spike in the dentate gyrus of urethane-anesthetized rats. We found that performance in a delayed-response task was significantly enhanced in the presence of electrical LC activation. These results suggest that the electrophysiological changes observed in the brainstem may play a role in mediating the behavioral effects of LC activation. Further studies are needed to determine the mechanisms underlying these effects and to understand their implications for the treatment of neurological disorders.

**Response from Neuronal Systems:** We have investigated the responses of neurons in the region of the lamb tonsil that harbors the pontine swallowing nucleus. Neurons were characterized by their responses to orthograde and retrograde stimulation with chemical, mechanical, and thermal stimuli. We found that neurons responsive to orthograde stimulation were longer-lasting than those responsive to mechanical and thermal stimuli. These findings suggest that orthograde stimulation may be more effective than mechanical and thermal stimuli in stimulating the pontine swallowing nucleus.

**Reference:**


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**Further Reading:**


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**Conclusion:**

Overall, our findings suggest that the use of orthograde stimulation in the brainstem may be a promising approach for the treatment of neurological disorders. Further studies are needed to investigate the mechanisms underlying these effects and to develop more effective methods for stimulating the brainstem.
BRAINSTEM SYSTEMS

BRAIN STEM INHIBITION IN ADAPTATION
J. Zabara. Temple Univ. Sch. of Med., Phila., PA 19140
A vestibular model of adaptation is utilized to test the hypothesis that inhibitory processes in the brainstem determine the extent and rapidity of adaptation to a changing environment. Brainstem inhibition is initiated by a vagal afferent mechanism which is activated by electrical stimulation of specific nerve groups. Four conscious and unrestrained adult male and female squirrel monkeys of Bolivian origin were exposed to 30 rpm horizontal rotation in a transparent plastic test chamber for up to 2 hours/day for 6 to 12 days. Rotation was sustained for the entire session lasting from 15 to 120 minutes with multiple vomiting episodes permitted. Vomiting latency and frequency were measured in both stimulated and non-stimulated animals. Implantation of a vagal cuff is performed on monkeys anesthetized with sodium pentobarbital IV (35 mg/kg). A pair of adjacent contacts is used for bipolar stimulation at 1-10 ma amplitude, 4-100 Hz frequency and 0.3-0.6 msec duration. Electrode connections are made with contacts housed in a nylon receptacle cemented to the top of the skull. Each monkey had a substantial reduction in emetic response to rotation during vagal activation. The percentage reduction in each of the four animals was 81% (#1), 88% (#2), 72% (#3) and 83% (#4). The average percent reduction for all animals was 81%. Thus, adaptation can be enhanced by a vagal afferent mechanism incorporating inhibitory transmitters.

Evidence for glutamic acid decarboxylase and GABA receptors within rat IP suggests a neurotransmitter role for GABA in this nuclear complex. In the present study, antiserum against GABA-glutaraldehyde-keyhole limpet hemocyanin was used to localize GABA-like immunoreactivity (GABA-LI) in rat IP. Rates were colchicinized and perfusion fixed (4% paraformaldehyde, 0.5% glutaraldehyde, 0.5% K2Cr2O7 in 0.05M PO4 buffer, pH 6.5). Vibratome sections (50µm) were processed by the avidin-biotin-peroxidase method. Staining was blocked by 100µM GABA-glutaraldehyde conjugate but not by 500µM conjugated glutamate, glycine, or taurine. GABA-LI neuronal somata were abundant in rostral (R), dorsomedial (DM), central (C), intermediate (I), and dorsal lateral (DL) subdivisions of IP, but found infrequently in the caudal lateral (CL) nuclei and absent from the apical (A) subnucleus. GABA-LI processes were present in all IP subnuclei but prominent in IP-RL, I and A. Quantitative evaluation of some cross-sectional areas indicated that GABA-LI somata were among the largest of all neurons in IP-DM and -R, but among the smallest of cells in the remaining subnuclei. Results are consistent with the hypothesis that GABA has a neurotransmitter role in IP function, and suggest that the influence of IP GABA-LI cells may be extrinsic as well as intrinsic to this nuclear complex.

Injections of the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) in substantia nigra (SN) or ventral tegmental area (VTA) produce labeling of extensive descending pathways. Injections in VTA can fill the entire mesencephalon as well as the entire pons, with anterogradely labeled varicose axons and terminal fields. In sagittal sections, terminal fields can be seen throughout the midline raphe nuclei and as far caudal as the pontine reticular nucleus. A more discrete distribution to the forebrain projecting raphe nuclei, dorsal and median raphe, as well as to raphe pontis, is seen when the injection site is in lateral VTA and medial SN. Anterogradely labeled axons are in the order of 0.5 µm in diameter. Few descending projections are seen in tegmentum from SN pars reticularis injections although ipsilateral superior colliculus is extensively labeled with axons arranged in a pattern of pillars and laminae that surround blank holes.

The extent of labeling from SN and VTA is greater than that reported for dopaminergic connections, implying that a substantial portion of these descending axons are non-dopaminergic. It should be born in mind, however, that these injections have been made in areas that carry heavy descending traffic and so test to the fullest extent possible the assertion that PHAL does not label fibers of passage.
Supported by USPHS Grant NS20841.

The existence of a cholinergic projection from the pedunculopontine tegmental nucleus (PPT) in the midbrain to the cholinergic basal nuclei of Meynert in the forebrain has been suggested (Wolf & Butcher, 86, Sema et al., 86). In order to determine whether the cholinergic neurons of the nucleus basalis receive input from a cholinergic source such as the PPT for the laterodorsal tegmental nucleus (LDT), or from noncholinergic neurons in the mesopontine tegmentum, we used rats treated with the following combinations of techniques: (1) CHAT immunocytochemistry only, (2) anterograde transport of PHAL from the region of PPT to label midbrain afferent terminals in the forebrain, combined with CHAT immunocytochemistry to label cholinergic basal nuclei neurons, and (3) anterograde transport of PHA-L from the region of the PPT, with retrograde transport of WGA-HRP from the cerebral cortex to label cortically-projecting nucleus basalis neurons.
We found that: (1) Cholinergic somata and dendrites (both distal and proximal) in the nucleus basalis of Meynert did not receive synaptic input from CHAT-immunoreactive synaptic terminals. However, numerous non-CHAT-immunoreactive terminals did contact cholinergic dendrites, and cholinergic terminals did contact noncholinergic dendrites; (2) Axons labelled with PHA-L from the mesopontine tegmentum did not form synaptic contacts onto cholinergic somata or dendrites in the basal forebrain, but did contact noncholinergic neurons in this region; and (3) PHA-L labelled axons did not form synaptic contacts onto somata retrogradely labelled from the cerebral cortex, but did synapse onto non-retrogradely labelled neurons in the basal forebrain. These results suggest that there is little, if any, cholinergic input from the PPT or LDT onto the cholinergic basal forebrain neurons. Supported by PHS HD-04583, PHS NS-17681(BHW) and PHS ST32 GM-07281(AEH).
CHEMICAL SENSES: GUSTATORY PATHWAYS


Decerebrate rats exhibit satiety and normal oro-facial reactions to tastes, but do not respond to the challenges of dehydration or sodium depletion. Nor do they exhibit the ability to acquire or maintain conditioned taste aversions (CTAs). To explore the feasibility that taste cell activity is modified by CTA learning in these preparations, responses were recorded from 42 single neurons in the nucleus tractus solitarius (NTS). The area of NST was paired with LCI-induced malaise. Results were compared with those reported previously for unconditioned decerebrates (Mark & Scott, '84). When neurons were categorized in sweet and non-sweet responsive groups, responses evoked by the CS from sweet-sensitive cells were shown to be increased by 16%. Post-stimulus time histograms revealed that this increase was due largely to a spike of activity, three times greater than unconditioned decerebrate levels, which peaked 950 ms after stimulus onset. Multidimensional scaling analyses, however, did not reveal a shift in the coding of the CS following conditioning. Whereas stimulus profiles evoked by the CS and quinine became more similar with CTA development in intact animals, these profiles remained distinct in decerebrates. These results demonstrate that a subset of NST neurons are functionally altered as a result of CTA learning independent of forebrain influences. The absence of a change in coding of the CS in decerebrates, however, may account for the fact that these animals do not demonstrate CTA learning behaviorally.

473.2 CHRONIC RECORDING IN AND AROUND THE HYPOGLOSAL NUCLEUS DURING INGESTION AND REJECTION OF Sapid STIMULI IN THE RAT. J.B. Travers and L.M. Jackson*. Dept. Oral Biology, Ohio State Univ., Columbus, OH 43210

The neural substrate for ingestion and rejection is organized in the caudal brain stem (Grill & Norgren, '78). Gustatory stimuli influence the motoneurons producing ingestion and rejection primarily over polysynaptic pathways (Mark et al., '83). We have begun to examine the neural circuitry underlying these responses by examining gustatory elicited responses of ingestion and rejection neuron in the caudal hypothalamic nucleus and the adjacent reticular formation. Under Nembutal anesthesia, rats were implanted with fine wires in the anterior digastic (AD), styloglossus (STG) and thyroarytenoid (PHA) muscles. In addition, a chronic microdrive was positioned over the hypoglossal nucleus and secured to the cranium. Wires were brought to an Amphenol connector for subsequent attachment to the instrumentation. Intra-oral cannulas were also implanted for the delivery of gustatory stimuli. Rats were tested in a Plexiglass observation chamber. Unit activity was recorded in response to the delivery of a battery of gustatory stimuli. During an ingestion response, rhythmic bursts of unit activity were either in phase with the anterior digastic (protuber type activity) or out of phase with the AD and in phase with STG (retractor type activity). During rejection responses to QHC1, some neural responses showed a decrement, in contrast to the peripheral musculature. Supported by NS 24889

473.3 SODIUM DEPRIVATION PRODUCES ALTERATIONS OF CHORDA TYMPANI TERMINAL FIELDS IN THE NUCLEUS OF THE SOLITARY TRACT IN ADULT RATS. Camille Tessitore* and David L Hill (SPON: P. Lasiter). University of Virginia, Charlottesville, VA.

Recording from rat chorda tympani (CT) nerves have demonstrated that sodium deprivation on or before 8 days postconception and throughout development reliably produces taste responses to NaCl while taste responses to NH4Cl and KCl are unaffected. In order to investigate whether the alteration of the peripheral innervation is anatomically reflected in the first order synapse in the CNS, we examined terminal fields of the CT in Na deprived and Na replete animals. HRP was applied to cut nerves and the tissue was processed according a modified TMB technique after 24 hour survival. Results indicate that while the fields of both control and deprived animals were located in the rostral pole of the NTS, control CT terminal fields were confined in a discrete ovoid configuration whereas terminal fields of deprived animals were in a diffuse circular configuration. Preliminary results indicate that the total volume of terminal fields is increased in Na deprived animals as compared to controls. These findings indicate that the central projection areas of the gustatory system are sensitive to environmental manipulations. (Supported by NIH NS24741 & NS01215)

473.4 PARABRACHIAL NEURAL ACTIVITY DURING GUSTATORY STIMULATION IN AWAKE RATS. H. Nishiio and R. Norgren, Department of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033. Rats were adapted to receiving water while in a plastic restrainer. Under anesthesia, cranioplastic acrylic was attached to their skulls and formed around 4 pins that were bolted to the stereotaxic earbars. Two intracerebral electrodes were implanted. Ten days later the animals resumed a deprivation regimen in which they received their water while restrained and with their heads held securely by the acrylic cylinder isolated in the dorsal pens with microelectrodes introduced via a previously prepared skull opening. Data were collected from 46 cells in two animals while they responded to a standard stimulus consisting of 0.05 ml of 1.0 M NaCl, 0.3 M sucrose, 0.003 or 0.01 M citric acid, and 0.0001 M quinine HCl, as well as to water. For a sample of neurons, conditioning response functions were generated for some (n=7) or all (n=9) of the 5 stimuli. When compared with equivalent infusions of distilled H2O, 40 neurons responded differentially to one or more of the stimuli. The majority of neurons were most sensitive to NaCl (n=26) or sucrose (n=9). Within these categories, 7 cells responded to a single NaCl stimulus (Na4, S=3). Responses to citric acid and quinine were less frequent, sometimes confounded with apparent water responses, and except at highest concentrations, generally of lower amplitude. Supported by PHS grants NS 30397 and MH 00653.

473.5 ORAL SENSORY RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT (NST) RECEIVES PRIMARYafferent terminations from at least 6 separate gustatory receptor subpopulations, as well as somatosensory afferents from both the lingual and oral cavity. Single-unit responses were recorded from an extensive area of NST to ascertain the organization of responses arising from all oral sensory systems. Both gustatory and mechanical stimulation of oral structures were recorded from 17 gustatory-14 mechanically-responsive NST neurons; it is unclear where these NST cells project. However, the mechanical stimulation of oral structures, but none of the mechanical neurons were gustatory-sensitive. Seven gustatory cells received information from a single gustatory receptor subpopulation, such as the foliate papillae, whereas 7 others were activated by stimulating multiple receptor groups; e.g., the soft palate and foliate papillae. The remaining cells were sensitive only to mechanical stimulation of the entire oral cavity. Similarly, some mechanosensitive neurons were activated only by stimulating a specific subclass of papillae, whereas others were sensitive to both lingual and palatal stimulation. There was an orotopic map of both gustatory and somatosensory projections, with the somatosensory representation lateral and/or caudal to the gustatory. Supported by NS24884 and NH00653.

473.6 ANTERIOR TONGUE TASTE AND TACTILE PROJECTIONS TO NUCLEUS OF THE SOLITARY TRACT (NST) IN SHEEP FETUSES AND LAMBS. C. H. Mistrata. Univ. of Michigan, Ann Arbor, MI 48109. To determine whether there is a functional microorganization for salt taste and tactile projections from fungiform papillae on the anterior tongue to second order neurons, and whether the organization changes during development, multunit recordings were made in the NST of sheep, 55 fetuses at 147 days (term - 147 days), six perinatal animals (5 days before or after birth), and six postnatal lambs (30 to 60 days) have been studied. With an initial location in the anterior tongue taste projection, neural activity was recorded at 0.25 mm steps throughout rostral-caudal and medial-lateral coordinates. Within each electrode track, responses were recorded in microanatomic areas 0.25 and 0.50 M NH4Cl, NaCl, and KC1, and light touch with a glass probe. At all ages there was a trend for NaCl to elicit larger responses relative to HCl, at more rostral NST coordinates. However, a well defined chemotopy for salts was demonstrated. Responses from papillae on the tongue tip contributed proportionately more to the taste response at more rostral NST locations. Throughout the nuclei, responses to NaCl were recorded most dorsally, then responses to salts and touch, and then, most ventrally to HCl. For tactile responses, the posterior tongue was represented most dorsally and the ventral tongue most ventrally. These data indicate some somatotopy for anterior tongue taste and tactile projections in NST that is established early in development, and the absence of any well defined chemotopy for salts. (Supported by NIH Grant NS 25825.)
473.7

CHEMICAL SENSES: GUSTATORY PATHWAYS

Since the early 1970s it has been known that female rats prefer higher concentrations of sweet stimuli compared with males. Recent data from our lab (D'Incrocci & D'Incrocci, 1985) have shown that in the parabigeminal nucleus of the PONs (PN) of ovariectomized female rats showed larger responses to sweet stimuli compared with PN units in males. Because it has been shown that the PONs have a dorsal preference for saccharin compared with intact females, it is possible to predict that responses to sweet stimuli in the PN of ovariectomized rats might be of lower magnitude compared with those in intact females. To test this hypothesis, we performed electrophysiological recordings of the 4 basic taste qualities in the PON of ovariectomized rats. Multidimensional scaling analyses of PON unit responses to the stimuli constructed a "taste space" that closely resembled that constructed by similar analyses of PON responses in males. These results suggest that decreased saccharin preference in ovariectomized rats may reflect a less sensitive subunit of the taste components of the PONs. Further, these results provide evidence that taste responses in the PON are influenced by both the activation and organizational actions of ovarian hormones. Further analyses will focus on this issue in greater detail.

Supported by PHS Grant S07RR01749-12 to P.M. DiLorenzo.

473.9

MENTAL AUDITORY PERIPHERAL AND CENTRAL NEURAL CODING OF HUMAN TASTE QUALITIES. L. G. Schabert and J. E. Cooper (Sponsor: B. Bryant Morse) Chemical Sensory Center, 3700 Market Street, Philadelphia, PA 19104

The rat tongue is sensitive to both thermal and chemical stimulation. Peripheral nerve studies have revealed that the neural architecture of the chemical and thermal stimulus in peripheral tongue temperature fibers. To explore the neural coding of chemical and thermal signals, we employed both peripheral nerve fiber and cortical recording techniques. In cats, concentrations of NaCl, HCl, sucrose, and quinine were recorded in the PbN of ovariectomized rats. Gustatory stimuli included 3 concentrations of each of the following: NaCl, HCl, saccharin, sucrose and quinine. Preliminary analysis of 32 PbN units in ovariectomized rats showed that neural activity may be elicited by chemical and thermal stimuli in the PbN of ovariectomized rats. There was no compelling evidence to the contrary. In addition, the gustatory cortex, a fluorescent dye, RH795, (a gift from T. C. A. Market, Oxford, England. Supported by BRSG Grant S07RR01749-12 to R.K.)

473.11


Models of gustatory neural coding have been based primarily on recordings from the hindbrains of anaesthetized rodents. Where these models fail to predict human psychophysical data, three levels of ambiguity must be addressed, species differences (rodent vs human), relevant neural level (hindbrain vs cortex) and anesthetic effects. We have developed the capacity to record from single gustatory neurons in the insular cortex of the alert macaque. We now report on the responses of 41 of these cells to 2 monkeys to a representative array of 16 salts, 2 acids, 4 sugars and 4 bitter stimuli at moderate to high concentrations. The spontaneous rate was 5.4±4.4 s⁻¹ (range=0.5-25.5 s⁻¹). Of 65 trials (16 stimuli x 4 neurons) 42 (64%) elicited excitation (CI 39%) inhibition or 40% (13%) no response. The mean breath-of-turning coefficient was 0.80 (range=0.38-0.99). Correlations between pairs of stimulus profiles generally confirmed expected similarities (r(4gC-xCI=+0.95) and dissimilarities (r(fluC-xHC=+0.29)) of taste quality. There was no compelling evidence to the contrary. Further analyses will focus on this issue in greater detail.

Supported by research grant BNS 8514202 from the NSF.

473.12


Optical recording methods were used to detect hamster cortical activity in response to electrical stimulation (ES) of the anterior and posterior tongue. Following a cranectomy over the gustatory cortex, a fluorescent dye, R0975, (a gift from Drs. T. B. Cohen and A. Grimaldi) which changes its Fluorescence with changes in membrane potential, was internally applied to the cortex. Fluorescence changes were recorded by a photodiode array. Anterior tongue ES resulted in activation of a small, (900 x 500 μ m) discrete cortical area. Posterior tongue ES activated a separate, more caudally located cortical area; there was also a temporal difference; cortical activity latency following anterior tongue ES (8-16 msec) was significantly less than the latency of 90-150 msec following posterior tongue ES. Surrounding brain areas were not activated by anterior or posterior tongue ES. Bilateral stimulation of the chorda tympani eliminated cortical activity in response to anterior tongue ES but not to posterior tongue ES. In conclusion, it appears that a discrete area of the hamster cortex which responds to tongue ES has been identified with voltage sensitive dyes. This area is bounded ventrally by the rhinal fissure and bounded dorsally by the somatosensory cortex. This area can functionally be divided into two parts: an area activated by anterior tongue ES and an area activated by posterior tongue ES. These results suggest that information to the central cortical area is carried by the chorda tympani. Supported by NIH grant NS16993.
CHEMICAL SENSES: OLFACTORY PATHWAYS

474.1


The rat olfactory bulb has high levels of glycogen phosphorylase activity which mobilizes glucose from glycogen. In cortex, phosphorylase is activated by norepinephrine. We therefore examined the role of norepinephrine in the control of olfactory bulb glycogen breakdown.

Olfactory bulb slices were incubated with tritiated glucose, resulting in tritiated glycogen formation. Slices were then treated with test drug, homogenized, and glycogen was extracted and measured.

Norepinephrine induced a concentration-dependent breakdown of glycogen with an ED50 of approximately 300 pM. The published value of the ED50 for norepinephrine-induced glycogenolysis in cortical slices is three orders of magnitude higher, suggesting that the olfactory bulb is an extremely sensitive target tissue for this amine.

We will also present data concerning the adrenergic receptor sub-type(s) underlying this effect. In particular, we will test the hypothesis that the sensitivity of olfactory bulb phosphorylase to norepinephrine is the result of an alpha-receptor mediated potentiation of a beta-receptor mediated response.

474.3

EVIDENCE FOR GABA-MEDIATED INHIBITION IN THE SALAMANDER OLFACTORY BULB. E. A. RADCLIFF, R. R. NEFF and J. S. KAUER. Neurosciences Program and Dept. of Neurosurgery, Tufts-New England Medical Center, Boston, MA 02111.

In the olfactory bulb, GABAergic interneurons, many of which are granule cells, exert powerful control over the excitability of mitral/tufted output cells. In response to electrical and odor stimulation, this control is manifest as a feedback inhibition mediated through periglomerular dendrodendritic synapses. As a first step toward understanding how odor responses are generated in the olfactory bulb, we have used unit recording techniques to determine if GABAergic, granule-layer cells might control mitral/tufted cell excitability in the tigeral bulb.

Intracellular recordings obtained from mitral/tufted cells in the salamander have shown that a prolonged biphosphatase, similar to that in the rabbit and turtle, follows orthodromic stimulation (Kauer, J. Neurophys. 59: 1988). Immunocytochemical staining has also provided evidence that granule-layer cells, as well as other interneurons are GABAergic. The effects of these cells on mitral/tufted cell activity has also been assessed using GABA blockers during video-rate imaging of voltage-sensitive dye fluorescence (Kauer, Nature 311:146, 1988).

We are now applying the combination of these methods to the analysis of how other components of the olfactory bulb circuits might contribute to the generation of responses elicited by odor and electrical stimulation. Supported by grants NS-22035, NS-20003 and the Dept. of Neurosurgery.

474.4

EARLY AND LATE RESPONSES IN SLICES OF SALAMANDER OLFACTORY BULB: OPTICAL RECORDING OF ELECTRICAL EVENTS THAT DEPEND UPON CA++. A. P. CLAYTON and B. M. SALZBERG. Univ. of Penn., Phila., PA 19104.

Fast and slow extrinsic optical signals have been reported in several olfactory preparations. The former reflects the compound action potential, but the origin of the slow signal is less certain. Optical recordings (Baclofen, 0.5-5mM) from tigeral bulb slices with Ca++ at 0.5-5mM generated an additional long-latency optical signal. The slow signal that followed stimulation of the glomerular layer was reduced in size by Ca++ (0.1-1mM), and in the presence of low Ca++ replacement, while replacement of Ca++ by Sr++ increased its size and duration. Neither the fast nor the slow optical signals observed at the level of the mitral cell and granular dendrites, were abolished by TTX (5µM) or 0[Na+]. These observations, together with the results of experiments using GABA, Baclofen, and APV, suggest the presence of a long-lasting enhancement at mitral cell dendrites which depends upon Ca++ and which gives rise to the slow optical signals. Supported by NSPS grant NS 16824 and a Fogarty Fellowship to A. P. Claytton.

474.5

HIGH RESOLUTION VIDEO IMAGE OF ODOR RESPONSES IN THE SALAMANDER OLFACTORY SYSTEM. J. G. RAUSCH and R. R. NEFF. Psychology Program and Dept. of Neurosurgery, Tufts-New England Medical Center, Boston, MA 02111.

Global events simultaneously occurring in neuronal elements that have been activated in parallel can be measured by video-rate imaging of voltage-sensitive dye fluorescence (Kauer, Nature, 1988). Using this method, patterns of electrical activation in the tiger salamander olfactory bulb have been observed. In the present study, we show an improvement in temporal resolution from 33 to 10 ms/video-frame and report an analysis of responses obtained after odor stimulation of the olfactory receptors. Stimulation with one-half second presentations of a single, orthogonal pattern of depolarization and hyperpolarization in different bulbar layers which are similar to those seen in the same region using intracellular recording. The distribution of activity within the layers is distinctly different for different odors. These data provide additional information in excess of that obtained from the patterns of activity distributed in time and space across the cells at each level of the olfactory pathway and have begun to quantify the assessment of the activity of the patterns across animals. Initial experiments are in progress in which we have imaged activity in the olfactory receptor cell population after both odor and electrical stimulation.

Supported by grant NS-20003 and the Dept. of Neurosurgery.

474.6


Acquisition of conditioned responding and learned odor preferences during olfactory classical conditioning in rat pups requires forward pairings of the conditioned stimulus (CS) and the unconditioned stimulus (UCS), i.e., the CS must precede and overlap temporally with the UCS (Sullivan 4: 1988). Other temporal patterns of CS-UCS pairing were tested for a behavioral odor preference to the CS. Wistar rat pups were trained (PN1 - PN18, 10 min/day) with either forward (odor) or backward (stroking then odor) CS-UCS pairings. On PN19, pups were tested for a behavioral odor preference to the CS. Only pups with forward CS-UCS pairings demonstrated modified behavioral and neural responses to the CS. Thus, these results suggest that, as for the behavioral responses, modified olfactory bulb neural responses are specific to associative learning. Formation of this neural association requires temporal constraints on the CS - UCS relationship. [Supported by NS26100 to RNS, BNS8660876 to DAW and ML, and MH00371 to ML]

Olfactory delta during postnatal development results in profound structural and neurochemical modification of the olfactory bulb. The present report is a description of the neurophysiological characteristics of the olfactory delta. Wistar rat pups had a single naris occluded at 2 days after birth. At 20 days postnatal, when the olfactory bulb had been anesthetized, the occluded naris was reopened, and the previously opened naris was closed. The functioning of the primary olfactory bulb, the olfactory tracts, and the lateral olfactory tract was monitored by recording single and multi-unit activity. The results suggest that although levels of granule cell-mediated inhibition appear immature after early deprivation, the deprived bulb is functionally capable of responding to odors. [Supported by grants MH09635-01 to KMG, and BNS8606786 to DAW and MLA]
ROLE OF ASSOCIATION FIBERS IN SELECTIVE LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX. J. S. Stripling and D. K. Patneau. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the olfactory bulb (OB) or lateral olfactory tract (LOT) elicits an evoked potential in the pyriform cortex (PC) whose initial wave (period 1) reflects activity of OB PC-pyramidal cells via the LOT. Subsequent excitation within the PC. This is followed by period 2, which is associated with inhibition of PC pyramidal cells. Repeated high-frequency stimulation of the OB produces a selective long-term potentiation (LTP) of period 2 (Brain Research 464: 281-291, 1988), while stimulation restricted to the LOT does not (Soc. Neurosci. Abstr. 12: 508, 1986).

In the present experiment male Long-Evans rats with chronically implanted electrodes received repeated high-frequency stimulation of either the LOT, layer Ib, or layer III of the PC. Stimulation of either layer Ib or layer III produced a selective LTP of period 2 in the PC similar to that produced by OB stimulation in previous studies. Stimulation of the LOT produced minimal effects. These results suggest that activation of the association fiber system which runs in layers Ib and III of the PC is critical for the production of LTP. An accompanying presentation (Patneau and Stripling) will present a model of LTP in the PC which incorporates these findings. (Supported by NSF Grant BNS 85-19700.)


Corticotropin releasing factor (CRF) is present in fetal sheep neurons as early as 90 days gestation (d GA) and projections to the external zone of the median eminence are dense by 132 d GA (Levidiotis et al., Neuroendocrinology 46: 453, 1987). Activity of the CRF mRNA in the hypothalamus is highest from 130 to 145 d GA, suggesting that further maturation of the CRF system takes place at this time. The aim of the present study was to examine the organization of corticotropin releasing factor (CRF) immunoreactive structures in the fetal sheep hypothalamus at this critical period of development.

Six Rambouillet-Columbia sheep fetuses, delivered by cesarean section under Bio-Tal anesthesia at 130 to 145 d GA, were perfused with Zamboni's fixative and their brains processed for localization of CRF. CRF, or neurophysin (NP) using the ABC method. CRF and NP within the magnocellular hypothalamic nucleus and the hypothalamo-neurohypophyseal tract were well developed. There were fewer CRF cells than CRF neurons and they were often segregated. Projections from the PVN joined the fibers from the SON and accessory nuclei in the hypothalamo-neurohypophysial tract to the median eminence and posterior pituitary. CRF and NP fibers of the internal zone of the median eminence continued into the neural lobe of the posterior pituitary, a zone contains a dense plexus of CRF axons and scattered CRF axons. Some CRF and NP fibers extended into the pars tuberalis. Few CRF fibers could be further traced into the pars disitalis, where they appear to terminate in association with ACTH cells. Within the pituitary stroma, scattered CRF and NP axons extended into the intermediate lobe. The role of CRF in ADH and oxytocin release, CRF at the adult stage, but their role within the adenohypophysis may provide an alternative or synergistic mechanism in the control of ACTH secretion apart from the neural-hormonal route. Supported by NIH Grants RO-1 HD 18418, PO-1 HD 21550 and RO-1 AM 16166.
2478.3 

PROLACTIN DOES NOT EFFECT POSTNATAL BRAIN DEGENERATION IN THE PRETERM RABBIT. A.H. Lecomte* R. R. K. Hooper, J. H. Wratten, K. G. Finan, T. K. Winston and V. C. Ross Dept. of Neurosurgery, Children's Hospital, and Brigham and Women's Hospitals, Boston, MA 02115.

Postmatures. A striking sexual dimorphism in the pattern of growth hormone (GH) secretion, however, the role of the CNS in mediating this sex difference is unknown. In the present study, we determined the involvement of hypothalamic GH-regulatory peptides, somatostatin (SRIF) and GH-releasing factor (GRF). In freely-moving male rats, the GH response to a single 60 min iv GRF iv was significantly greater at peak compared to trough times, the latter due to antagonization by the cyclic increased release of SRIF. In contrast, females failed to exhibit a time-dependent difference in GH responsiveness to GRF suggesting that the pattern of hypothalamic SRIF secretion in females does not follow the male ultradian rhythm. Passive immunization with a specific antibody to rGRF obliterated spontaneous GH pulses in both males and females; moreover, in females anti-rGRF attenuated GH trough levels indicating a physiological role for GRF in maintaining the elevated GH baseline of females. Hypothalamic immunoreactive GH content was significantly lower in females compared to males (58.0±20.6 vs. 79.2±75.9 pg/fragment; P<0.02). Conclusions: The sexual dimorphism of GH secretion is likely due to different temporal patterning of hypothalamic GRF/SRIF signals to somatotropes with females exhibiting tonic, rather than episodic, SRIF secretion compared to males.

2478.4 


... functions in the LHRH neuron independently of maturational age. The TX leads to a comparable reduction in LHRH release and content; this occurs in both age groups. ADX leads to a marked reduction in LHRH neuron to a given steroid.

478.7 

PLASMA TESTOSTERONE DELAYS ONSET OF MATERNAL BINOCULAR VISION IN HUMAN INFANTS. R. Held, J. Bauer, and J. Gilverda, M.I.T Infant Vision Laboratory, Cambridge, MA 02139.

The ages of onset (average 3.5 months) of stereopsis and the fusion/rivalry-discrimination measured behaviorally are delayed by several weeks to months compared to females. In contrast, no sex differences are found in the developmental course of grating acuity. This suggests that it is visual mechanisms rather than biological level delay the maturation in males. Indeed, it is during this period of development when radical changes in neuronal connectivity are occurring in the visual cortex: synaptogenesis is at its peak rate and the segregation of the ocular dominance columns is in progress. Just prior to this period, the average levels of testosterone (T) rise to a peak (about 8 weeks of age) in males and then subside. T-levels in females of this age are almost zero. To assess the role of T in mediating the sexual differences in the ontogeny of the LHRH neuron, a number of experiments were performed to determine if the ablation of T could be delaying the maturation of LHRH neurons in males. Initially, the ablation of T leads to a comparable reduction in LHRH release and content; this occurs in both age groups. ADX leads to a marked reduction in LHRH neuron to a given steroid.

2478.8 

IN VIVO UPTAKE OF 3H-ESTRADIOL BY THE FETAL PRIMATE BRAIN. R.P. Michael, R.W. Bonas® and H.D. Rees. Dept. Psychiatry, Emory Univ. School of Medicine, and Georgia Mental Health Inst.1256 Briarcliff Road NE, Atlanta, GA 30306.

Neurons in the preoptic area, hypothalamus, and amygdala of fetal macaques have been shown to be responsive to estradiol. However, the in vitro administration of 3H-estradiol, and a major portion of the nuclear radioactivity in these regions is in the form of 3H-estradiol. To study the uptake and binding characteristics of estradiol in fetal primates, 3H-estradiol was administered via the umbilical vein to rhesus and cynomolgus monkeys (2 males and 3 females) on day 122-125 of gestation. The fetus was removed from the mother 24 hours later by cesarean section. The brain was frozen for thaw-mount autoradiography and samples of cerebral cortex were used for analysis of radioactivity in nuclear and supernatant fractions by high-performance liquid chromatography. There were only a few weakly labeled neurons in the hypothalamus and amygdala of one female, and no labeled cells in the brains of the remaining four fetuses, despite estradiol dosages of 1,000 ng/kg. This finding may be due to the fact that the absence of labeled neurons in autoradiograms might have been due both to rapid clearance and an inability to pass from plasma to cytoplasm and, hence, into the nucleus. The method preventing the entry of estradiol into neuronal nuclei may protect the fetal brain from any deleterious effects of estradiol hormones during development. (Supported by MH 40430 and by the Georgia Department of Human Resources.)
DEVELOPMENT OF SEXUALLY DIMORPHIC AXON NUMBERS IN THE LARYNGEAL NERVE OF XENOPUS LAEVIS. Darcy B. Kelley and Jane Dennis*. Department of Biological Sciences, Columbia University, New York, N.Y. 10027.

In classical anuran craniofacial plasticity, the dimorphism in form and behavior occur during development that are paralleled by changes in the nervous system. It is likely that amine and certain peptide plays important roles in the development of the nervous system. In the laryngeal nerve, the axons that innervate the vocal folds are dimorphic.


In the lobster, Homarus americanus, dramatic changes in form and behavior occur during development that are paralleled by changes in the nervous system. It is likely that amine and certain peptide plays important roles in the development of the nervous system. In the laryngeal nerve, the axons that innervate the vocal folds are dimorphic.

478.11 DIFFERENTIAL EFFECTS OF ESTROGEN ON SUBSTANCE P mRNA LEVELS IN THE RAT ANTERIOR PITUITARY AND HYPOTHALAMUS. E.B. Brown, R.E. Hartig, A.J. Krause. Dept. of Anatomy & Neurobiology, Washington University Medical School, St. Louis, MO 63110; Dept. of Anatomy, Tufts University School of Medicine, New Orleans, LA 70112.

Gonadal steroids alter substance P (SP) peptide levels in the rat anterior pituitary (AP). Estrogen decreases and androgen increases AP SP in male and female rats. In order to determine whether these steroid effects occur at the level of peptide synthesis, we analyzed preprotachykinin (PPT) mRNA levels in the AP of male and female rats. SP mRNA levels in the AP were increased by estrogen and decreased by androgen. Furthermore, this regulation is extended to the hypothalamus, where the estrogen effect on SP levels is at least in part due to changes in peptide synthesis. Furthermore, this regulation is extended to the hypothalamus, where the estrogen effect on SP levels is at least in part due to changes in peptide synthesis.

478.12 TRANSMITTER UPTAKE, STORAGE, SECRETION AND METABOLISM III.

479.1 RECONSTRUCTION OF THE ATP-DEPENDENT GLUTAMATE UPTAKE SYSTEM INTO LIPID MEMBRANES. N. Carless*, P. Kish* and T. Ueda. Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109.

We have previously provided evidence for ATP-dependent glutamate uptake into synaptic vesicles, supporting the neurotransmitter role of glutamate. Based on the unique properties of the vesicular uptake system, we have proposed that the vesicular glutamate uptake is a key step in glutamate uptake into synaptic vesicles.


We have recently reported that bovine chromaffin cells contain IGF-I receptors (N.K. Dahmer and R.L. Perlman, J. Neurochem. 61:312-317, 1990). We have now examined the effects of IGF-I on catecholamine secretion from these cells. Chromaffin cell cultures were maintained in serum-free medium in the presence of insulin (10 nM). Insulin also enhanced catecholamine secretion, but was much less potent than IGF-I; thus, the action of IGF-I is probably mediated by IGF-I receptors that mediate post-synaptic secretion of catecholamines, and that mediate post-synaptic secretion of catecholamines (3 μM), suggesting that it acts at a step distal to Ca2+ entry. IGF-I appears to be an important regulator of noradrenaline and adrenaline secretion (Supported by NIH grants K04563, HL04775 and HL20205).
479.3
CHOLINERGIC SYNAPTIC VESICLES CONTAIN TWO ATPASE ACTIVITY T. K. Parsons, L. Manzoni, and K. Nordeen, Dept. of Chemistry, University of California, Santa Barbara, California 93106.

A glycoprotein ATPase has been purified from Torpedo californica electric organ synaptic vesicles and shown to have a native-detergent solubilized Mr of 210,000 ± 9000 composed of 110, 104, 96, and 98 kDa subunits. The ATPase is inhibited by vanadate (K_i 10 μM) and it accounts for 2/3 of the total activity in the vesicles. Acetylcholine activator transport is not inhibited by vanadate; rather, it is stimulated by 25 percent. Vanadate-insensitive ATPase transport is inhibited by 10 μM Acetylcholine activator. This second activity presumably is due to the vacuolar-type ATPase generally postulated to pump protons into most secretory vesicles. The glycoprotein ATPase purified by a form of a technique immediately with the ATPase in question, but the ATPase is ouabain insensitive and not stimulated by Ca^2+. Other properties are being investigated.

479.4
SODIUM-DEPENDENT UPTAKE OF NUCLEOSIDES BY DISOCIATED BRAIN CELLS FROM RAT. H. D. Gillette, Dept. of Pharmacol., Univ. of Manitoba, Winnipeg, MB, R3E OM3.

Nucleoside transport was studied using a mixed population of dissociated brain cells from adult rat. Accumulation of [3H]adenosine during brief (15 sec) incubation periods was significantly enhanced by the presence of 110 μM sodium. This sodium-dependent uptake was saturable at concentrations that ranged from 0.25 to 100 μM. Kinetically, the rapid accumulation of [3H]adenosine was best described by a two-component model (KD = 0.9 μM and 8.9 μM/pmol/mg protein/15 sec, and for the low affinity component 313 μM and 3427 pmol/mg protein/15 sec, respectively. In the absence of Na^+, the E_max value was significantly higher: 1.8 μM. [3H]Adenosine accumulation was best described kinetically by a one-component system that in the presence of Na^+, had E_max and V_max values of 1.0 μM and 2.6 μM/pmol/mg protein/15 sec, respectively. As with [3H]adenosine, in the absence of Na^+, the E_max value was significantly higher: 1.8 μM. Sodium-dependent transport of [3H]adenosine was inhibitable by ouabain and 2,4-dinitrophenol. Nucleosides demonstrated high affinity and selectivity in blocking the sodium component. Thus, high affinity sodium-dependent nucleoside systems, in addition to facilitated diffusion systems, exist on brain cells from adult rats.

479.5
LOCALIZATION OF THE Dopamine UPTAKE SITE IN RAT BRAIN USING ([3H]-BTPC. J. K. Kamely, H. Huss*, and F. Filloux, Dept. of Psychiatry, Univ. of Tennesee, M-I, UT. 84132.

To date, a number of tritiated ligands have been used for autoradiographic labeling of dopamine(DA) uptake sites in the brain. These include ([3H]-mazindol, ([3H]-nomifensine, ([3H]-methylphenidate and ([3H]-GBR12935. None have proven satisfactory thus far (Babishlhiophenylol-1-cyclobutylaminopropionyl-1-Cyclohexyl)N-Piperidin (GK-13 or BTCP) has been shown to be a potent inhibitor of DA uptake whose affinity for noradrenergic uptake sites is substantially lower (Chicheportich et al. We have found ([3H]-BTPC binding to 10μm coronal sections of rat forebrain to be both reversible and saturable and of comparatively high affinity (K_d = 10-1250). The highest specific binding was achieved using 50μM Tris buffer (pH=7.0) which also contained 120μM NaCl. Under these conditions, nonspecific binding was < 1% Preliminary autoradiograms demonstrate that specific binding in the forebrain is concentrated in the basolateral and olfactory tubercle, while little or no specific binding is present in the cerebral cortex. Specific binding is potently displaced by GBR12909, a selective DA uptake inhibitor. No greater degree of displacement is achieved by mazindol, an agent with greater affinity for the NE sites than for DA uptake sites. These data suggest that ([3H]-BTPC should be very useful for autoradiographic studies of the dopamine transport complex.

479.6

Imipramine and other tricyclic antidepressants inhibit the active uptake of serotonin into neurons and blood platelets. This effect involves high-affinity binding of imipramine to a portion of the uptake site where the inhibitor appears to act allosterically. A reduced number of imipramine binding sites in a subset of depressed patients may reflect differences in serotoninergic tone between these patients and normal subjects. The possibility that an unidentified endogenous ligand normally regulates serotonin uptake at this site and may play a modulatory role in the serotonin system is under investigation in our laboratory. We have previously reported an inhibition of imipramine binding by extracts of human urine. This inhibitory activity has now been further purified using TLC on silica plates with a mobile phase of acetonitrile/methylene chloride/water (59/1/2,0). The activity was first concentrated from the urine using a reversed phase C18 extraction column (Baker-T 00 SPE), eluting with acetonitrile. In a typical urine sample, 50% inhibition of [3H]imipramine binding was achieved with extract from 0.1 ml of urine (assay volume = 0.5 ml). Eluates from 3 adjacent regions of the TLC plate had inhibitory activity. Reverse phase HPLC of each of these eluates shows 1-3 major and a small number of minor uv-absorbing species. Complete purification and identification of the active ligand(s) seems near.

479.7
COORDINATE STIMULATED RELEASE OF CALCIUM IN GENE-RELATED PEPTIDE (CGRP) AND SUBSTANCE P (SP) FROM DISOCIATED CELLS OF NEONATAL RAT VAGAL 03000 SENSORY NEURONS. D. H. Maclean, Div. of Embryology, Brown University, Rhode Island Hospital, Providence, RI 02902.

These studies sought to determine whether in cultures of vagal sensory neurons the stimulated release of two neuropeptides, SP and CGRP, is differentially regulated, and whether they are co-released during a single event. Neonatal rats were sacrificed at 10 days gestation and cultured in vitro. Neuropeptide content was measured by RIA in release media (KBE, 18 FBS) from 15-20 min after 2-4 weeks in culture. Both SP and CGRP release were stimulated by capsaicin. 34 μM capsicain stimulated RIA and CGRP release 6±10x above basal levels, e.g., SP, 23±9 pg/well and 68±20 pg/well, and CGRP 91±27 pg and 702±22 pg/well, basal vs. capsaicin = 6±9 and 2±2. The maximal effect at 10 μM, resulted in 3-4x basal release e.g., SP, 6±13 pg and CGRP 290±49 pg/well, but did not further enhance capsaicin release. Capsaicin did not raise basal or BK-induced release. Serotonin (0.5 μM) weakly stimulated basal release and enhanced BK-evoked release e.g., CGRP 180±55 vs. 455±167 pg/well. Both SP and CGRP media content were 2.5-3.5x basal levels; amphetamine did not enhance basal or BK stimulated release. Conclusion: Both CGRP and SP are released from vagal sensory neurons in response to similar stimuli.

479.8
THE DISTRIBUTION OF HIGH AFFINITY UPTAKE SITES FOR [3H]GABA IN THE HUMAN MYENTERIC PLEXUS. A. Krantiatia, A. Kallili, and C. Krause, Digestive Diseases Research Group, Dep't of Physiology and Div. of Gastroenterology, Univ of Ottawa, Ottawa, K1H 8M5, Canada.

GABA is a transmitter of myenteric neurons in the guinea-pig, and is proposed to be an entero-neurotransmitter in the rat, cat, and human, where GABA and its metabolic enzymes have been localised in neural plexus of the intestine wall (Tanaka 1985, Life Sciences, 37: 2221-2235). However, the disposition of GABAergic neurons in the human myenteric nerve system is unknown. Therefore we sought to determine the occurrence and disposition of high affinity uptake sites for [3H]GABA in the human myenteric nervous system. Autoradiography (after Krantis et al. 1986, Neuroscience 17(4): 1243-1253) was performed on paraffin sections (12-18 μM) of human myenteric plexus taken at surgery and fixed with 50% formalin/Ammonia. [3H]GABA, 5.10^-10 to 10^-4 M, in the absence or presence of specific inhibitors of high affinity GABA uptake. Radio labelled GABA was accumulation of myenteric neurons in all segments examined. However, few labelled processes could be seen. These results show that GABA is transported into human myenteric neurons by a high affinity system.

Funded by the Medical Research Council of Canada.
TRANSMITTER UPTAKE, STORAGE, SECRETION AND METABOLISM III


EXCITATORY AMINO ACIDS X


480.2 EXPRESSION OF THE N-METHYL-D-ASPARTATE/PCP RECEPTOR IN XENOPUS OCYCIES INJECTED WITH mRNA FROM NCB-20 CELLS. L. Kushner*, J. Lemna*, M.V. Bennett and R.S. Zakin Albert Einstein College of Medicine, Bronx, NY 10461

The n-methyl-d-aspartate (NMDA) receptor complex is a ligand-gated cation channel which contains regulatory binding sites for Mg2+, Zn2+ and glycine. Recent evidence suggests that this receptor complex also contains the phenylalkylamine (PCP) receptor binding site which may result in a psychomimetic effects of PCP derivatives, opiates and the dioxalanes, and that PCP acts as a blocker of the NMDA gated channel. The mouse neuroblastoma-Cortical-Cholinergic (NCC) of rat brain has a PCP receptor similar to the rat brain receptor as is demonstrated by complexion receptor assays under equilibrium binding conditions. This site was labelled by the N-ethylmaleimide-N-(1'-C-thienyl)cyclohexyl]piperidine (TCP) with a binding affinity (Kd) of 335 nM and a receptor density (Bmax) of 9264 fmo/l/mg protein. In order to investigate the molecular outline of the NMDA/PCP receptor of NCB-20 cells we looked for its expression in Xenopus oocytes injected with NCB-20 cell poly(A)RNA (50 ng/cell) Oocytes were voltage clamped and perfused with Mg2+-free amphibian Ringer's solution. Drugs were bath-applied. At a holding potential of -60 mV, NMDA (10 µM, with 10 µM glycine) evoked a partially desensitizing inward current that was potentiated by glycine (EC50 = 0.1 µM) and inhibited by the competitive antagonist D-(+)

480.3 CANNOT FURTHER DISTINGUISH NMDA RECEPTORS FROM KAINATE RECEPTORS.
480.3
NMDA, KAINATE AND QUISSULATE RECEPTORS OF RAT BRAIN EXCITATORY AMINO ACID RECEPTORS EXPRESSED IN XENOPUS OCYTES: SUMMATION EXPERIMENTS. M.V. L. Bennett, J. L. Ernster*, L. Kushnir* and R.S. Zuckin (SPIN: E. Masurovsky). Albert Einstein College of Medicine, Bronx, NY 10467.

Rat brain mRNA injected into Xenopus oocytes leads to responsiveness to the glutamate agonists NMDA (N), kainate (K) and quisqualate (Q). To address this last question we are investigating the development and expression of excitatory amino acid receptors in cultured embryonic hippocampal neurones in culture also indicated that the inhibition of NMDA responses were abolished, suggesting a negative modulatory effect of 7-Cl KYNA at the glycine site. These findings support the view that PCP is an open channel blocker.

480.4
COMPETITIVE AND NON-COMPETITIVE BLOCK OF NMDA/PCP RECEPTORS EXPRESSED IN XENOPUS OCYTES. M.V.L. Bennett*, J. L. Ernster*, L. Kushnir* and R.S. Zuckin. Albert Einstein College of Medicine, Bronx, NY 10467.

NMDA receptors were expressed in Xenopus oocytes following injection of rat brain mRNA (Kushnir et al. PNAS 85: 3205, '88). Glutamate is required for responses to bath-applied NMDA. Responses to varying NMDA concentrations (with 10 µM glycine) (n=4, 600 µM) have a Hill coefficient, n=1 and Kd=1 µM. Imax increases with glycine concentration, but n and Kd are unaffected. APV (IC50=3 µM) is a competitive blocker; Kd is shifted to higher concentrations with little effect on I or I. Max. Block by PCP is non-competitive; Imax is reduced without change in K or n. PCP appears to be a channel blocker and block and unblock exhibit use dependence. PCP alone blocks very slowly; block develops rapidly in the presence of NMDA. Rate of onset of block increases with agonist concentrations (when channel open probability is higher), but degree of block depends on PCP and not NMDA concentration (indicating that PCP enters and leaves channels primarily if not exclusively when they are open). Removal of agonist leaves the channel blocked. Recovery is slow in the absence of applied agonist and is sped by reapplication. Block is voltage dependent. Blocking blocking in the presence of apamin and is sped by reapplication. Block is voltage dependent. Blocking blocking in the presence of apamin and is sped by reapplication. Block is voltage dependent. Blocking blocking in the presence of apamin and is sped by reapplication. Block is voltage dependent. Blocking blocking in the presence of apamin and is sped by reapplication. Block is voltage dependent.
480.9

Responses of voltage-clamped cultured chick spinal neurons to prolonged application of glutamate fade with a time course dependent on the receptor type and the method of application. Rapid application of 1 µM glutamate to outside-out membrane patches show that C2 (APV resistant) currents fade with a 1/2 of 5 ms. The concentration dependence of desensitization was studied by whole cell recording. Bath application of 2-100 µM glutamate (with 2-APV) reduced the responses to ionophoretic glutamate pulses applied at receptor hotspots with an ID 50 of 8 µM. This value is in the range of concentrations that bathe neurons in vivo and in vitro (2-15 µM). Significant reduction was observed with glutamate concentrations that produced little or no receptor activation. Indeed, superfusing cells with fresh, glutamate-free solutions offered increased the size of ionophoretic glutamate responses. Ionophoresis and spontaneous excitatory synaptic currents were reduced in amplitude by 485% (± S.D., N-4 cells) following application of 10 µM glutamate. These data suggest that receptor desensitization by normal concentrations of glutamate modulate the efficacy of central synaptic transmission. This work was supported by NS 16486 and the ALS Association.

480.10
CHARACTERIZATION OF A RAPIDLY DESENSITIZING GLUTAMATE CURRENT IN CULTURED POSTNATAL HIPPOCAMPAL PYRAMIDAL NEURONS. L.L. Tho, D.B. Clifford, and C.F. Zuraski, Washington University School of Medicine, Departments of Psychiatry and Neurology, St. Louis, MO 63110.

Rapid pressure applications of glutamate evoke a rapidly activating and rapidly decaying inward current in >95% of the cultrures. A PAT rec hippocampal pyramidal neuron was studied with the whole cell patch clamp technique. The decay occurs despite the continued application of glutamate, probably due to the rapid desensitization of the AMPA and kynurenic acid and APV. Supermaximal concentration of glutamate is able to partially desensitize the kynurenic acid and APV but not the APV induced reduction of channel opening frequency. The interaction between these compounds will be discussed.

480.11

Kainic acid (KA) (20-300 µM) and quisqualic acid (QUIS) (0.5-500 µM) stimulate the release of preaccumulated [3H]-d-aspartate from cultured cerebellar granule cells in a dose-dependent manner. This effect of KA, but not that of QUIS, could be antagonized by kynurenic acid (90-200 µM) or by 2-5 µM 3-amino-5-phosphonovaleric acid (APV) (500 µM). QUIS (10-500 µM) inhibited in a dose-dependent manner the KA-induced [3H]-d-aspartate release induced by KA+QUIS without antagonism by KYN. However, simultaneous application of ineffective concentrations of KA (10 µM) and QUIS (2.5 µM) evoked the release of [3H]-d-aspartate, and this release could also be blocked by KYN. Moreover, QUIS (5-50 µM) or KYN (50-200 µM) antagonized KA-induced comp formation half maximal concentrations were 1.0 µM QUIS and 100 µM KYN. These biochemical experiments were extended by complementarly electrophysiological studies. Whole-cell voltage clamp recording from cerebellar granule cells showed that currents generated by 30-100 µM KA were reversibly diminished by QUIS and KYN. Furthermore, KA-induced inward currents had a time constant of 81 ms. The current recovered completely in less than 4 s. The percentage of desensitization, the time constant of decay, and the time of recovery are identical at +50 mV and -50 mV.

480.12

Cultured neurons from the visual layers of the rat superior colliculus express a glutamate receptor-mediated component. This component can therefore be isolated in the compound response of tectal neurons to exogenous N-glu­tamate (Glu). Binding to QA-receptors probes up to half of the current elicited with 100 µM Glu. All collicular neurons from E21/F1 rats responded to QA, its EC 50 ranging from 0.1 to 0.3 mM. Application of QA modifies the response to other Glu-agonists. Testing QA with various concentrations of KA showed that QA acts as a competitive antagonist of KA. The effect of QA on subsequently elicited NMDA is more complex. The initial transient component of QA (NMDA) was suppressed, while the persistent component temporarily recovered from its block by APV. These experiments demonstrate, thus, a modulatory action of QA on NMDA-receptors. QA had a strong effect on GABA-release, even under the condition that voltage-activated Na and Ca currents were fully blocked. QA-induced depolarization of presynaptic terminals and/or liberation of Ca from intracellular stores by activation of second messenger chains may account for this novel effect of QA.
Oleic acid inhibits sham feeding when dose-dependently infused while total caloric intake NOT, G. Smith, M. J. Gibb. Dept. of Psychiatry, New York Hospital—Cornell Medical Center White Plains, NY 10605.

The specific CCK receptor antagonist L364,718 was used in the present study to block endogenous CCK satiety in Zucker rats. 6 male fa/fa obese and 6 male Fa/Fa lean rats were adapted to a 6 hour food deprivation, and IP injections 20 min before a one hour test meal of liquid food. Rats were injected with either L364,718 (0.375 mg/kg) or vehicle. Each rat was tested twice at this dose, the results pooled and significance determined by correlated T-test. Food intake was increased 25% in lean rats following L364,718 (19.6 ml±0.06) compared to vehicle alone (16.1 ml±0.54, t=0.72, p<0.01). Obese Zucker rats did not increase food intake following L364,718 (16.9±0.33) compared to vehicle (15.5±0.35, t=1.49, p<0.1).

The results suggest that blocked CCK receptors cause over eating in lean rats, while the blockade had no effect on obese rats, suggesting they do not release endogenous CCK. These data support an hypothesis of a failure of CCK satiety in the genetically obese Zucker rat.
FRIDAY AM
FEEDING AND DRINKING VIII

461.7

We have previously reported a small, transient rise in plasma insulin (i) prior to the transient decline in blood glucose (TDBG) that precedes meal initiation (MI) in free-feeding rats. We have also shown that following vagotomy, TDBG are not always followed by MI. In order to further characterize the role of i in MI, plasma I and meal pattern were correlated in chronically cannulated, weight matched, male Wistar rats with sham (5) or total subdia- phragmatic vagotomy (V). Blood was continuously withdrawn (25 µl/min for up to 100 min) from lightly heparinized awake rats and pooled over 4 min intervals. In experiments without MI, no significant changes in plasma I were observed over the sampling period in both S and V rats. In experiments (n=6) with MI in S rats, I rose to a peak (60%) then declined to a minimum level at 26 and 8 min prior to MI respectively. In contrast, in V rats I did not rise but instead gradually fell to a plateau prior to MI (n=6). These data demonstrate that a vagally dependent I spike is not necessary for MI. Since we observed an increased frequency of small TDBG that were just at or below threshold for normal MI in V rats, these data suggest that V may have two effects that result in less than the faithful coupling of MI and BG: 1) denervation of peripheral glucose receptors and 2) absence of 1 spike that may enhance the magnitude of TDBG.

461.9
VAGOTOMY ATTENUATES SUPPRESSION OF SHAM FEEDING INDUCED BY INTESTINAL INFUSIONS. Daniel R. You, H. Stokesberry and R.G. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

We examined the participation of the subdia phragmatic vagus nerve in the suppression of sham feeding induced by intraintestinal (IN) nutrient infusions or by intraperitoneal (IP) injection of capsaicin. Nutrient infusions or injections were made while rats were allowed to feed with an open gastric fistula. Results are as follows:

<table>
<thead>
<tr>
<th>IN or IP</th>
<th>Sham Vagotomy</th>
<th>Vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-8 (2 µg/kg)</td>
<td>51.27±2.7</td>
<td>10.03±5.7</td>
</tr>
<tr>
<td>maltose (0.13 kcal/ml)</td>
<td>27.95±1.4</td>
<td>4.75±0.9</td>
</tr>
<tr>
<td>oleate</td>
<td>63.29±1.9</td>
<td>1.9±10.3</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>55.21±4.6</td>
<td>21.7±8.0</td>
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</tbody>
</table>

Our results indicate that the subdia phragmatic vagus nerve is the predominant neural substrate mediating the suppression of sham feeding induced by IP CCK-8 or by IN maltose or oleic acid. Mucosal as well as vagal substrates are involved in suppression of food intake by L-phenylalanine. These results extend our previous findings using the neurototoxin capsaicin and suggest that small unmyelinated vagal sensory neurons mediate the suppression of feeding by intestinal chemical stimuli.

461.11
INTESTINAL (IC) ASPARTEATE DOES NOT INFLUENCE SHORT-TERM FOOD INTAKE (FI) OR SELECTION WHEN ADMINISTERED WITH A CARBOHYDRATE (CHO) LOAD. R.J. Bialik, B.T.S. Li and C.H. Anderson. Dept. of Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8.

High doses of aspartate (the methyl ester of phenylala-nine and aspartate) have been reported to alter postabsorptive feeding behavior in rats. In the present study we investigated whether aspartate would alter the normal feeding responses (i.e. FI suppression, diet selection) following a CHO load. Rats were adapted to a 12 h (1800-0600) feeding schedule, with a choice of low (L5%) and high (35%) protein diets available only during the light period. On different days, rats received either CHO or CHO plus aspartate by gavage. The CHO load (pure corn starch, 4 ml/kg of a 37.5 g/dl solution) reduced total FI (by 36%) and consumption from the low protein diet (by 43%) in the first hour, without affecting 2 or 12 hr FI. Addition of aspartate (0, 50, 200 or 500 mg/kg) to the CHO load failed to alter FI compared to CHO alone. We conclude that the reduction in total FI and the selective suppression of a low protein diet following a CHO load are unaffected by large doses of aspartate. (Supported by NSERC of Canada)

461.12

A role for peripheral and central glucose receptors in the control of meal initiation (MI) has been proposed. We have previously shown that exogenous glucose, infused during a transient decrease in blood glucose (TDBG), delays MI. Glucose and glucose analogs such as z-t-butyl-4-olide (2840) have been reported to decrease the firing rate of both hepatic vagal afferents and glucose sensitive neurons. The ability of 2840 to block MI was assessed in free-feeding female Wistar rats chronically implanted with cardiac and femoral cannulas. Rats were continuously recorded (up to 150 min) following a 1 min IV infusion of 10, 20, 40 or 80 µM of 2840. During intermeal intervals, changes in blood glucose were monitored. Ad libitum administration of 30, 40 and 40 µM (C5%) but increased by 30% following 80 µM; feeding was not observed in any of these 24 trials. When 2840 was superimposed on transient decreases in BG, MI was reliably blocked following 20 and 40 µM and only partially (33%) blocked following 10 µM. In contrast to glucose, the effect of 2840 was not altered by the shape of the decline in BG, suggesting that uncoupling of MI from BG occurred at the level of detection by glucose sensitive neurons. We conclude that 2840 uncouples MI from BG at low doses by blocking the detection of declines in BG and at higher concentrations by also altering BG dynamics.
EVIDENCE THAT THE CELLULAR SOURCE FOR RETINAL REGENERATION IN GOLDFISH IS WITHIN THE NEURAL RETINA

It is known that there can be functional regeneration in larval lampreys (Cohen et al., PNAS 83:7267-7266, 1986). We now show that adult goldfish share this capacity for regeneration, but incompletely.

Partial lesions of either lateral or medial tracts were made in young feeding adult lampreys (Petromyzon marinus and Ichthyomyzon ancistrius). After 8-10 months, the animals were tested for functional regeneration. The spinal cord was dissected out with the notochord and superfi cial with curase (15mg) and D-glutamate (0.25-0.50 mM) to activate fi citive swimming. The activity of two motor nerves, one rostral and one caudal, was recorded by silver wire electrodes. When stable, the recorded bursts were amplified and their cycles coordinated with the recording. The recordings were made over a period of 1-2 weeks. The results show that the spinal cord can regenerate after partial lesions, and that this regeneration occurs in a normal pattern of activity. The regeneration is also complete, as the lampreys can swim normally after the surgery. The regeneration is not perfect, however, as some movements are still impaired. In conclusion, the spinal cord of adult lampreys has the capacity for functional regeneration after partial lesions. The regeneration occurs in a normal pattern of activity and is complete, although not perfect. Supported by NSF grant BNS 86-06607 and CFI 86-50488.
EVIDENCE FOR REINNERRATION OF SKELETAL MUSCLE BY MOTOR-NEURONS VIA VENTRAL ROOT IMPLANTS INTO THE SPINAL CORD OF THE CAT. T. Carlgstedt, S. Cullheim*, H. Lind* M. Risling* and B. Ulfhake* Dep't of Hnsurgery, Dept of Anatomy, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden.

We have previously shown that cat lumbar motoneurons may reinervate ventral roots after division of their axons in the spinal cord (Risling et al. Brain Res 180:15, 1983). This unexpected regenerative capacity of the motoneuron has been further explored in cats after implantation of avulsed ventral roots into the transplanted cord.

The left ventral roots L6-S1 were avulsed from the surface of the cord. The L6 root or rostral L7 rootlets were immediately inserted into the ventral roots. The rats were killed at about 12 months and the spinal cords, corresponding to the root segments, were removed. Previous work showed that an undifferentiated cellular mass, the regenerative plaque, originates from the distalmost motoneuron remaining after division and gives rise to the new neuromasts. We identified the type of cell that initially seeds the plaque, and determined how the plaque advances during regeneration. DIC microscopy, combined with the decalcification of the cranial and spinal cord, revealed that cells on the posterior margin of the deepest layer of supporting cells in the last neuromast remaining after a tail cut became mitotically active about 6 weeks after the tail was amputated. Cell divisions were frequent in this region, which elongated medially and anteriorly, taking up the remaining neural tissue.
483.1
REGIONAL APPEARANCE OF MYELIN CONSTITUENTS IN THE DEVELOPING RODENT SPIRAL CORD. B.K. Forsberg, M.E. Schwab and F. Savio. Brain Research Institute, Univ. of Zurich, August-Forel-Str. 1, CH-8029 Zurich, Switzerland.

The regulation of oligodendrocyte differentiation and myelin formation is poorly understood at present. On the other hand, oligodendrocytes contain specific genes capable of inhibiting nerve fiber growth (Caroni and Schwab, '88). Studying the appearance of myelin specific glycolipids (GalC) in the rat cervical spinal cord we found that the time of expression of these components is highly specific for a given structure. The regional appearance of myelin constituents in the central funiculus (P1) and dorsal ascending tracts (P2-P4). Pyramidal tract (P1) and gray matter regions (patchy appearance) myelinate last. Frozen sections of spinal cords were used as substrates for cultured neuroblastoma cells and neurons. On adult and P13 tissue white matter areas were strongly inhibitory for oligodendrocytes; however, the expression of inhibitory substrates could locally influence nerve fiber growth.

483.3
ELECTRON-HISTOCHEMICAL OBSERVATION OF 5'-NUCLEOTIDASE IN PRIMARY CHICK EMBRYONIC NEURAL CULTURES. H.C. Ludwig, P. Shohami* and E. Markakis. Department of Neurosurgery, Georg-August-University, Robert-Koch-Str. 40, D-3400 Goettingen, Federal Republic of Germany.

5'-nucleotidase has shown in several investigations to be an ubiquitous enzyme present in neuro nal and neural progenitor cells and gliall plasma membranes (Kreutzberg et al., Brain Res. 158, 1978:247). The ectoenzyme may play an important role in neural-glial interactions in which glial cells are providing adenosine to the neurons and therefore facilitate neuronal growth, development and differentiation. Presently the expression of 5'-nucleotidase was examined in aggregating neurons and glial cells, isolated from 8 day old embryonic chick brain and retina in monolayer culture systems. By modification of known cytochemical methods for EM demonstration of 5'-nucleotidase we were able to define the enzyme on the plasma membranes of glial flat cells. Special interest was directed towards the heterogeneity of the membranes of retinal Mueller cells grown in histotypically oriented rosettes. In these rosettes the degree of heterogenous expression of 5'-nucleotidase coincides with developing neurons and retinal receptors.

483.5
EXPRESSION OF A 35-kDa SUBSTRATE FOR THE EGF RECEPTOR KINASE KINASE IN MUSCLE RAPHÉ OF THE FLAME PLATE PRIOR TO THE ARRIVAL OF DEGENERATING FIBERS IN RAT EMBRYOS. James A. McKanna* (SPON: J.A. Pulliam), Dept. of Cell Biology, Vanderbilt Univ., Nashville, TN 37232.

A 35-kDa protein (p35), purified by Java and Cohen (JBC 259:2636) as a preferential substrate for the tyrosine kinase activity of the EGF receptor, is developmentally regulated in embryos and is predicted to play a role in morphogenesis. In rat embryos of 11 days gestation (E11), p35 is expressed in a ventral midline raphé of primitive neural cells that run from the lumen of the neural tube to the pial surface. The p35 staining is first apparent in the rostral hindbrain, but by day E14 the entire raphé runs from the caudal edge of the midbrain to the tip of the spinal cord. Thus, p35 is expressed in glial cells at the future site of axon entry prior to the arrival of degenerating axons, and it may serve gradient, guide or gate functions. The p35 immunoreactivity disappears from the CNS by postnatal day 3; however silver stains show that the raphé cells persist through adulthood. Thus we predict the role of the transmembrane p35 calcium and phosphatase may influence the cytoskeleton. Supported by CA43720.

483.4
OMEGA-3 FATTY ACIDS IN THE DEVELOPING PHOTOSENSOR CELL. N. G. Bazan, F. Catalfamo, J. B. L. Scott*. LSU Eye Center, New Orleans, LA 70112.

The omega-3 essential fatty acid family comprises a major acyl group of retinal photoreceptor membranes and synaptic membranes of brain and retina. Docosahexaenoic acid (22:6w3) represents nearly 50% of the fatty acyl group of disk membranes of photoreceptors although its function is not well understood. We are studying the omega-3 fatty acid family in the developing mouse pup and find that the major omega-3 acid found in pups in mother's milk is linoleic acid (18:2w3). In addition we have studied the accumulation of 22:6 in developing photoreceptor cells and brain of mice at different postnatal ages and have examined the ability of these animals to synthesize 22:6 from 18:3.

We have found that the content of 22:6 in photoreceptor cells increased 3- to 4-fold in the various phospholipid classes as the cells synthesize the adult complement of rod outer segment disk membranes. Furthermore, studies of the synthesis of 22:6 from [14C]18:3 indicate that the liver is the major site of conversion in the developing pup and that the growing photoreceptor cells and to brain primarily in the 22:6 form. A similar model is postulated also to occur during the differentiation of synaptic membranes. Supported by EY0428 and the Edward G. Schlieder Educational Foundation.

483.6

Our study characterizes the early influence of the CNS on survival of neural crest intercalary cells. We found that basic fibroblast growth factor (BFGF), synthesized in the CNS, stimulates survival of a1R-1 immunoreactive NC cells segmented in vivo by a silastic membrane impregnated in 100 ng/mL BFGF. Rescued cells were then observed for over 30 hr, after grafting in 12 out of 16 cases all grafts survived. In contrast, in control PC groups no surviving cells could be found in any of 10 control embryos. BFGF was also tested on trunk NC cultured with unmeshed floor plate. In control, no surviving intercalary cells were found; in contrast, the survival of several NC cells was dependent on the number of BFGF-1 positive non neuronal cells in 1-day old cultures of 1.8 to 8.2 fold over controls unincubated at 10 ng/mL. Similar increase in the number of non neuronal cells was obtained when BFGF was added to pure NC cultures. BFGF had no mitogenic effect on mouse NC cell line but found to increase the incorporation of 3H Thymidine into acid insoluble material in somite cultures deprived of NC by 3.3 to 8.4 fold over control. Our results demonstrate a direct effect of FGF on survival and/or differentiation of the NC-derived non neuronal cell population. Sponsored by the MDA and The Israel Acad. of Sciences.
DIFFERENTIATION AND DEVELOPMENT VIII


A genomic chicken library was screened with human nerve growth factor (NGF) receptor cDNA probes at moderately stringency. Four positive bacteriophage clones were isolated and characterized by restriction mapping and Southern blot analysis. A 5.5 kb Hind III fragment was sequenced at one end of the human cDNA sequence. The region of homology was further localized, analyzed by DNA sequencing and found to correspond to the extracellular domain of the NGF receptor. Northern blot analysis of poly (A) RNA from embryonic chicken brain detected a unique and specific mRNA of 3.9 Kb in length, similar to the size seen in human melanoma cells, rat PC12 and Schwann cells. The same mRNA could also be detected in chick spinal cord and dorsal root ganglia. **

483.8 GABA AND GABA_A/BENZODIAZEPINE RECEPTOR-LIKE IMMUNOREACTIVITY IN THE DEVELOPING RAT CEREBELLUM. D.L. Miescke and P. Rakic. Section of Neuroanatomy, Yale School of Medicine, New Haven, CT 06510.

To explore the possibility that the development of local neuronal circuits may be related to the expression of neurotransmitters and their receptors, we have studied the ontogeny of GABA and GABA_A receptors in cells which form GABAergic inhibitory connections in the rat cerebellum. Cerebellar rat pups ages 0 days to 21 days post-natal were immunostained with GABA antisera (Immunocytological) and E9 monoclonal antibodies directed against the GABA_A/benzodiazepine receptor complex (Stevens et al., Mol. Brain Res. 87). Immunolabeled tissue was examined with light and electron microscopy.

The cerebellum of new born animals contains only pre-natally generated Purkinje and Golgi-axonal neurons. At this early stage, both cell types could be immunolabeled with GABA and E9 antibodies. The other cells of the cerebellar cortex arise post-natally from a germinal external granular layer which is present from about 3 to 21 days. Cells of the external granular layer and descending migrating neurons were not labeled with either GABA or E9 antibodies at any age. However, after arrival in the granular layer, maturing granule cells and their growing dendritic processes were E9 positive. Like granule cells, basket and stellate cells in the molecular layer do not appear to immunoreact with either antibody during their bipolar stage, and express E9 and GABA only after they develop dendrites and axons. These results indicate that cells which participate in GABAergic cerebellar circuits express GABA receptors only after they attain their final position and begin elaborating dendrites involved in synaptogenesis.

Supported by NIH grants NS14841, NS22880.


The trisomy 16 mouse is an excellent model for the human Down's syndrome (DS). It is postulated that sensitivity to interferon may underlie defects in neuronal formation in DS (J Neuro Sci 1987, 79:91). When applied to cultures taken from normal fetal mice, interferon increases immunohistochemical expression of the 210K neurofilament subunit. This effect can be blocked by the application of oxyphenbutazone which inhibits interferon- mediated metabolic pathways. CNS cultures taken from trisomy 16 fetal mice express greater intensity of the 210K neurofilament subunit than normal cultures, while application of oxyphenbutazone normalizes trisomy 16 CNS neurofilament expression.***EM observations were made of the cortical plate within the developing telencephalic vesicle at E17. The results included: 1. increased neuronal microtubular profiles which were more coiled and curved in the trisomic condition than in normals; 2. increased neural nuclear membrane irregularity in trisomic neurons; 3. a decrease in the number of neurites in the cortical plate from a normal number of neurites in the normal mouse and this number normalizes trisomic conditions; 4. a significant increase in the number of neurites in the normal condition. 5. Observations concerning nuclear morphologic differences in trisomy 16 may be related to the related differences in nuclear histone expression in Alzheimer's disease.

483.10 A BEHAVIORAL AND ANATOMICAL INNER EAR MURTANT GENERATED BY INSERTIONAL MUTAGENESIS IN TRANSGENIC MICE. E.B. Crenshaw III*.


Schwann cells (SC), which do not divide in vivo, proliferate in cell cultures when mitogenic signals, such as neurite or myelin breakdown products are present. In Tmbrer mutation, characterized by hypomyelination and basal lamina defects, the Schwann cells proliferate continually. The increase of SC is regular in the peripheral nerve from 3 day-old mice (+25%) to 4 days old mice (+60%). We were interested to analyse the proliferation capacities of Tmbrer and control SC when they are deprived of neural influence and after they are re-associated with nervous tissue. In cell cultures, SC from 1 and 4 days animals had a similar nuclei labeling index at each time tested (1 to 9 days in culture). By contrast, when Schwann cells were plated from 15 days old mice, Tmbrer SC cells proliferate less than control cells. Similarly, in wallerian degenerating sciatic nerve, the proliferation of Schwann cells is approximately 5 times higher in control than in Tmbrer.

When the tibial nerve is sutured to the degenerated distal stump, the SC proliferation is stimulated anew, less in Tmbrer than control. In culture, Tmbrer and control nerve extracts, added respectively to control and Tmbrer SC stimulate similarly their proliferation at a very high level. The Tmbrer mutation affecting primarily the SC provides a good model system to analyse the cellular origin and molecular nature of in vitro and in vivo mitogenic signals.


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Single rat nerve fibers were dissected and voltage clamped to study the properties of their K channels. K currents were recorded in cell-attached mode of Ringer's fluid and after acute paranodal demyelination with lyssolecithin and pronase. Results: 1. The analysis of tail currents recorded in isometric KCl solution revealed that K channels with slow gating kinetics were located in the nodal membrane whereas those with fast kinetics were located in the paranodal. The conductance of the fast K channels increased linearly with the increase in the axonal area which had been demyelinated. 2. The sigmoidal steady state activation curve of the slow K channels had an inflection point at -70 mV, i.e. 50% of these channels were in the open state at the resting potential. At negative potentials they showed an inward rectification which could be reduced by addition of 4-AP. 3. The paranoadal fast K channels were activated at more positive potentials and were selectively blocked by 1 mM 4-aminopyridine. Conclusion: Myelination in rat nerve is accompanied by a local segregation of slow and fast K channels.


We have previously identified several types of voltage-sensitive K+ channels in cell-attached recordings from the somatic and dendritic region of cerebellar Purkinje neurons in culture. In the present series of experiments, we have focused on the characterization of two of these channel types which were recorded in outside-out and inside-out membrane patches. Using saline solutions mimicking physiological conditions, the single channel conductance was found to be 50 and 75 pS. Both channel types were active at potentials depolarized to the normal resting membrane potential of these neurons (-60 mV) and had extrapolated reversal potentials of 30 and -15 mV, respectively. The potential for Cl- by ion substitution did not alter the reversal potential for the unitary events: changing the charge for K+ potential for K+ did, demonstrating that the channels were K+ selective sensitive to low concentrations of extracellular TEA, channel activity being significantly reduced by 1 mM concentrations. The activity of both channel types was also reduced by 10 mM extracellular Ba2+.

484.3 SINGLE POTASSIUM CHANNELS IN CULTURED RAT HIPPOGLIAL NEURONS. J. G. McLennan, Dept. of Pharmacology X Therapeutics, The University of British Columbia, Vancouver, B. C., V6T 1W6, Canada.

The properties of voltage-dependent potassium channels in cultured rat hippocampal neurons have been studied using the patch clamp technique. With the inside-out mode and 140 mM K+ in the bath and 5 mM K+ in the pipette a channel with conductance 150 pS commonly observed after depolarization steps. This channel is selective for K+ since the extrapolated reversal potential is close to that predicted for K+ and shifts to a value of 0 mV at the bath solution contains 5 mM K+; the channel was non-conducting when the solution contained 140 mM Cs+. Single exponential fits were adequate to describe the histograms of tail currents for the K+ channels with membrane depolarization acting to increase the channel open time. The distributions of closed times required two-component fits reflecting short closed sojourns during open events and longer closed periods between openings. A concentration of 10 mM TEA, applied to the outside of the cell membrane (outside-out mode), blocked the 52 pS channel. In the cell-attached mode with no TTX in the solutions, outward K+ currents were associated with the after-hyperpolarization phase of the action potential. The properties of the K+ channels studied are consistent with those associated with the delayed rectifier K+ conductance and would serve to mediate excitatory potentials in hippocampal neurons. (Supported by B. C. Health Care Research Foundation.)

484.4 REPETITIVE FIRRING IN HIPPOCAMPAL NEURONS IS MODULATED BY THE MEMBRANE POTENTIAL PRIOR TO ACTIVATION, THROUGH THE ACTION OF A SLOWLY INACTIVATING POTASSIUM CURRENT, IP. J. F. Storm, Institute of Neurophysiology, University of Oslo, Oslo1, Norway.

In hippocampal neurons, the firing current is regulated by spike-activated K+ currents (IK, h and h). In addition to this "feedback" discharge control, there may be a "feed-forward" control by the membrane potential prior to activation of the cell (MP). Both currents are expected to modulate the initial firing, in ways which depend on the degree of activation (Ih) or inactivation (IA) at rest. Here I report evidence that a third potassium current, Ii, mediates a phystol by the MP in subsequent discharge. Voltage clamp measurements indicate that Ih inactivates more slowly and is more sensitive to 4-aminopyridine (4-AP) than Ii but it activates faster than Ih. (Storm, 1988, Bihphys. J. 51: 144A.)

CA1 pyramidal cells (n=36) in rat hippocampal slices, were impaled with KCl-filled micro-electrodes, at 31-35°C. Repetitive firing was elicited by injecting long depolarizing current pulses (1-10 s). The MP was reduced by steady current injection. At MP close to the normal resting potential, there was often a 200-800ms delay to the onset of firing, while the cell depolarized slowly, forming a "ramp". The ramp and delay appeared to reflect inactivation of I, because: 1) the input resistance increased during the ramp; 2) if the ramp was blocked by 1 mM AP; 3) hyperpolarization of the MP (to enhance I,) increased the delay (up to 10s); whereas 4) depolarization (which inactivates I,) abolished the delay and also appeared to counteract the spike frequency adaptation, which was reduced by hyperpolarization and enhanced by depolarization. Prolong firing frequency against current intensity (10-pulse), normally show a low-gain range ("primary range") for small intensities. This range was increased by hyperpolarization and abolished by depolarization of the MP, suggesting that I, is essential for this low-gain part of the I,f relation. (Supported by the Norwegian Research Council, NAVF.)

484.5 PHENCYCLIDINE BLOCKADE OF VOLTAGE-DEPENDENT POTASSIUM AND CALCIUM CURRENTS IN ISOLATED HIPPOCAMPAL, NEURONS. A. J. Yol, and R. R. Hume. Dept. of Biological, Univ. of Michigan, Ann Arbor, MI 48109.

Adenosine 5'-triphosphate (ATP) has recently been shown to elicit two distinct currents in embryonic chick skeletal muscle (Hume and Thomas, Soc Neurosociol Abstr 13:900); a rapidly activating, desensitizing current which has a reversal potential near -15 mV, and a more slowly activating current that reverses at the potassium equilibrium potential. The latter current is due to an increase in the permeability to potassium, but the early current might result from an increase in the permeability to various combinations of cations or anions. We performed ion substitution experiments in order to add the ions that are permeable during the early response to ATP. We found that ATP increases membrane permeability to anions as well as monovalent and some divalent cations, but not to larger ions like tetrachloroethylene and glucuronate.

We determined the reversal potential of the early response to ATP in different external solutions, and used the Goldman equation to calculate permeability ratios. There was little difference in the permeability to the monovalent cations sodium, potassium and cesium. On the other hand, larger cations like histidine were much less permeable. In addition, the divalent cations magnesium, calcium, and barium permeate in low, but not in ATP. Finally, we found evidence that indicated that small anions such as chloride and acetate were also permeable, while the larger anion, glucuronate was much less so. We are now testing the possibility that the cation and anion permeability may be through a single charge nonselective ion channel activated by ATP.
A large positive shift in the peak potential of the AD occurred when external calcium was replaced by glucuronate. However, the AD disappeared when external calcium was lowered from 1 to 0.1 mM. Replacing external calcium with barium resulted in barium spikes following depolarization, but no AD. When cobalt replaced calcium, no regenerative responses were present. In these experiments, the sodium spike was blocked with tetrodotoxin. With external cobalt but no calcium, the AD could often be restored by including 30 mM calcium in the 3 M KCl recording electrode. This effect could not be mimicked with magnesium.

Whole cell patch clamp recordings were made from myoblasts under conditions in which chloride was the only plausible charge carrier. Large currents were observed following depolarization in approximately half the cells when internal calcium was buffered at 10^{-6} M, but currents were absent or greatly attenuated at 10^{-4} M. The reversal potential for these currents depended on EC_{50} and activation and inactivation were both voltage sensitive.
MUSCARINIC ACTIVATION OF IP3 MEDIATED CHLORIDE CURRENT IS INHIBITED BY THE 9-A-BUNDT SUBUNITS OF G-PROTEINS. T.M. Moriarty, B. Gilchrist, D. Goodale, R. Stuehr (Departments of Neuroscience and Pharmacology, Mt. Sinai School of Medicine and Bronx V.A. Medical Center, New York, N.Y.)

This study examines the mechanism of G-protein coupling of receptors to IP3 production. The ACh response was examined in two-electrode voltage-clamped oocytes in a superfusion apparatus. Modulation of the MACh evoked Cl-current was examined in oocytes microinjected with 10 nM ßγ heterotrimers purified from both rod and human RBC. The presence of a G-protein in oocytes was shown by pertussis toxin (PTX) labeling of a 40 kDa band from oocyte membranes. A 46 kDa band was labeled by an antisem specific for the ß-subunit. PTX treatment uncoupled the MACh receptors from activation of the Cl-current. Cells microinjected with 1.5 ng of PTX showed a 95% reduction in the MACh activated Cl-current. Cells injected with equivalent volumes of Pt storage buffer showed no change in response. Cells injected with bovine serum albumin or reduced-subsunit also showed no reduction in response. Cells co-injected with varying concentrations of PTX showed a dose-response relationship with half-maximal inhibition of MACh activated Cl-currents at about 1 nM. Cells injected with 1.5 ng of PTX could not respond to both applied agonist, but could generate the Cl-current on intracellular injection of IP3. These results suggest that there is a G-protein responsible for MACh receptor mediated signal transduction through IP3.

Center for Neurobiology and Behavior, Columbia University, 722 W 168th St., NY, 10032. Center for Experimental Biology, 222 Maple Ave, Shrewsbury, MA 01545. Studies in Aplysia have implicated CAMP-dependent protein kinase in several instances of synaptic plasticity including short and long term facilitation in synaptic neurons. To assess whether we have been using these proteins we have characterized the cDNA clones encoding catalytic (C) subunits of the enzyme.

A random cDNA library was screened with a mouse Cα cDNA (provided by G.S. McKnight). Nuclear acid sequence analysis of the clones revealed two different classes of cDNA encoding putative C isoforms. Both contained open reading frames 1550 nucleotides long, but differed at 39 positions between residues 423-549. The inferred amino acid sequences indicated that the isoforms were 97% identical, differing at 1042 positions between residues 142-183. The Aplysia amino acid sequences were 83-85% identical to murine and bovine Cα and Cβ, and 89% identical to the Drosophila subunit. Sequence analysis of an Aplysia genomic DNA fragment and 51 nucleotide mapping of cellular RNA confirmed that this variation arises through alternative splicing of two mutually exclusive exons homologous with and equal length to exon 6 of the mouse Cα gene. In contrast, 51 analysis of mouse brain and liver RNA using a Cα probe failed to show alternative splicing in this region. Although not strictly tissue specific, the Aplysia transcripts were expressed at different relative levels in different tissues. For example, the major transcript in neurons was present at relatively low levels in oocytes.

Recently, clones for the regulatory (B) subunits of the kinase have been obtained (Bergold et al., These Abstracts). Next, we intend to use nuclear acid and immunologic probes to investigate how combinatorial expression of B and C isoforms might be involved in synaptic plasticity.


Dopamine (DA) is a putative inhibitory neurotransmitter in some synapses in Aplysia. When applied on Aplysia pleural sensory neurons in culture, it produced membrane hyperpolarization and conductance increase by activating the potassium current. While acting through distinct D2-like receptors (as indicated by the effects of selective D1 and D2 agonists and antagonists), DA mimicked the inhibitory action of the peptide transmitter FMRFamide, which is thought to be mediated by the production of lipoxigenase metabolites of arachidonic acid. DA may stimulate the same cascade, as (i) inhibition of phospholipase activity with p-bromophenacyl-bromide prevented the actions of DA and FMRFamide; (ii) blockade of the lipoxigenase pathway with nordihydroguaiaretic acid markedly reduced the actions of both transmitters, while (iii) blockade of the cytochrome pathway with indoxyl ethacin had no effect. (iv) DA exerted its action in neurons loaded with cyclic-AMP analogues, indicating that it does not act by inhibiting adenylate cyclase. Thus D2-like receptors mediating inhibition in Aplysia sensory neurons may be linked to potassium S-channels by lipoxigenase metabolites of arachidonic acid.

508.11 CALCIUM DEPENDENT EFFECTS OF MAITOXIN ON PHOSPHOLIPASE D ACTIVITY AND ON CYCLIC AMP ACCUMULATION IN PC12 AND NCB-20 CELLS. Fabian Gagovsky, Takeshi Yamamoto and John W. Daly, LBC, NIDDK, NH, Bethesda, MD 20892.

The marine dinoflagellate toxin ma itoxin (MTX) stimulates phospholipase D activity in two systems: in a Ca2+-dependent calcium channel blocker resistant fashion. In PC12 cells the maximal stimulation of phospholipase D activity occurs at 1.5 mM Ca2+ [Kb], whereas in NCB-20 cells the maximal stimulation is observed at 3.5-4.5 mM Ca2+ [Kb]. Phospholipase D activity leads to the inositol phospholipids and to diacylglycerides. The latter through stimulation of protein kinase C can lead to a calcium-dependent, calcium channel blocker resistant facilitation in PC12 cells. The MTX-induced stimulation of phospholipase D activity in NCB-20 cells also increases cyclic AMP accumulation in PC12 cells and to inhibit receptor-mediated cyclic AMP accumulation in NCB-20 cells. MTX did potentiate forskolin-induced accumulation of cyclic AMP in PC12 cells. The effects of MTX on accumulation of cyclic AMP were calcium-dependent and the concentrations of calcium required for cyclic AMP generation was the same as the ones required for maximum stimulation of phospholipase D activity.

The results confirm previous studies on the heterogeneous input of protein kinase C to cyclic AMP generating systems performed with phorbol esters and demonstrate the utility of MTX as a unique tool for studies of systems that involve second messengers generated through stimulation of phospholipase D breakdown.

508.12 PHOSPHATIDYLCHOLINE TURNER AS A REPORTER FOR THE ACTIVATION OF DISTINCT CELLULAR SIGNALING PATHWAYS: IMPLICATIONS FOR THE ACTION OF NMF AND CAPSAICIN. S.L. Patterson* P.A. Gatti and M.R. Basili. "Dept Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA and MRC Molecular Neurobiology, Hills Rd, Cambridge CB2 0QH, UK.

The turnover of membrane phosphatidylcholine (PC) shows both constitutive and pharmacologically-stimulated components in a neuronal cell line NG115-401 which can be followed by the extracellular release of the radioactive metabolite. Although independently regulated, both constitutive and stimulated release occur through phospholipase D, releasing phosphatidylcholine. The basal release is reduced in the presence of agents that raise intracellular calcium, and by CaM-stimulating or calcium blockers. These findings do not appear to be specific to choline-esterase mediated turnover. However, the detailed pharmacology of the stimulated component indicates that NMF and capsaicin may be additional and independent stimulants of phospholipase D. Experimental design for the study of phospholipase D and phospholipase A2 was presented, which suggests that both NMF and capsaicin may activate novel or novel kinase(s) in these signaling pathways.

* Correspondence to: S. L. Patterson.
PRESYNAPTIC MECHANISMS II

486.1 TIME COURSE OF FUSION PORE CONDUCTANCE DURING EXCITOSIS OF NAST CELLS. \( ^{*} \) A. Spence, W. Almers, Dept. Physiol., U. Washington, Seattle, WA 98195

Excitosis begins with the opening of a fusion pore that connects the cleft to the active zone (Frederiksen-Nielsen, Almers, Nature 328:814). When the pore first opens, a transient outward current through it (trace 1) equilibrates the potentials across vesicle (\( E_v \)) and endoplasmic membranes (\( E_m \)).\( E_v - E_m \) is obtained by subtracting the time integral of I from its final value and then dividing by the vesicle capacitance. The pore conductance (trace G) is given by \( I/(E_v - E_m) \). G rises abruptly to a value \( G_0 \) (arrow); the rise time (\( 10^2-14 \) & 3D @ 10 MHz) is not well resolved. Later, G grows more gradually as the pore dilates. Sometimes (12% of cases) the slow phase is too rapid to be distinguished from the fast. The histogram of \( G_0 \) is skewed (range 80-800 pS, peak 200 pS, median 270 pS, 19 transients). The abrupt opening of the pore, faster than in gap junction channels (Neyton & Trautman, J. Exp. Biol 124:193; Veenstra & del-Rio, Am J Physiol 232:H170), is reminiscent of the abrupt opening of single leakage channels. NIH grants AR17803 and ON 39520.

486.2 TIME COURSE OF TRANSMITTER RELEASE AND CALCIUM CHANNELS AT THE MOLLE MOTOR NERVE TERMINAL. A. J. Bain* and D. M. J. Quastel, Dept. of Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

Following nerve stimulation the quantal components of EPPs are sharply focused in time with most appearing in a period of about 0.4 ms. Using a computer to locate each quantal and mini-EPP, we find that changes in release probability and latency of EPPs produced by repetitive stimulation in the presence of Ba are consistent with a fixed underlying time course of intracellular Ca at release sites, with each time constant being the same for the rising and falling phases. Brief submaximal focal depolarizations of nerve terminals (NTDs) elicit quantas with a more dispersed latency range than Ba. EPPs increased again to at least 100 ms and no inhibition of ion entry except by pulses much longer than those causing maximal activation. By extrapolation from results with Ba we find that the component of the EPP should persist even in the absence of ion entry and, with buffered Ca or with ion entry inhibited by benzamidone thrombin, there is no occurrence of small EPPs that grow with high frequency stimulation, in proportion to mini-EPP frequency. Thus, the influence of release and B. W. Edmonds, E.R. Kandel and M. Klein*, Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, New York, NY 10032.

Calcium current was recorded in isolated Aspura sensory cells using the whole-cell version of the patch-clamp technique. Currents obtained over a range of test voltages suggest the presence of two distinct components; a largely non-inactivating current that begins to activate around -20 mV and an inactivating current which is recruited at higher voltages (> 0 mV). A pharmacological separation has been achieved with nifedipine and FMRFamide. Nifedipine selectively antagonizes the non-inactivating component of current, whereas FMRFamide selectively reduces the inactivating component. Because FMRFamide is a transmitter mediating presynaptic inhibition in sensory cells, selective modulation of the inactivating component of current may be important in enhancing of K conductance for the expression of this form of plasticity. Unlike FMRFamide, nifedipine does not inhibit transmitter release. Taken together, the data are consistent with the idea that the two components of current only the inactivating component is important for transmitter release and for at least one form of synaptic plasticity.


We have examined the roles of presynaptic membrane potential and intracellular calcium activity in triggering the release of acetylcholine at an inhibitory synapse formed between two isolated somata from the small, well-isolated cell population of the superior cervical ganglion. Neurons B5 and B9 were co-cultured under conditions which suppress neurite outgrowth and permit formation of a soma-soma synapse. Synaptic transmission was monitored as miniature inhibitory postsynaptic potentials (mIPSPs) and miniature calcium currents in cell B5 recorded using whole cell patch clamps. Presynaptic membrane potential was controlled by simultaneous whole cell patch clamp of neurons B5. Presynaptic internal calcium concentration was controlled by internal perfusion of B5 with the photolytic chelator Nit-5, 75% loaded with calcium. We observed: 1) Depolarization of B5 to above +15 mV releases transmitter in a normal-calcium medium. 2) Nifedipine-stimulated release is reduced by presynaptic introduction of Nit-5, and abolished in a zero-calcium medium. 3) Photolyzing Nit-5 releases pre-synaptic free calcium from 240 nM to over 1.5 μM in the presynaptic terminal. Release increased to the rate caused by normal action potentials as calcium concentration rose to 100 μM, which is near the level occurring at release sites during action potentials. 4) Transmitter release is independent of presynaptic potential in the range -120 to -40 mV, when internal calcium was elevated by Nit-5 photolysis and external calcium was eliminated. We conclude that action potentials normally release transmitter solely by admitting external calcium to release sites, with no direct effect on secretion. Supported by NIH Grants NS 15114 and NS 24233.


Calcium current was recorded in isolated Aspura sensory cells using the whole-cell version of the patch-clamp technique. Currents obtained over a range of test voltages suggest the presence of two distinct components; a largely non-inactivating current that begins to activate around -20 mV and an inactivating current which is recruited at higher voltages (> 0 mV). A pharmacological separation has been achieved with nifedipine and FMRFamide. Nifedipine selectively antagonizes the non-inactivating component of current, whereas FMRFamide selectively reduces the inactivating component. Because FMRFamide is a transmitter mediating presynaptic inhibition in sensory cells, selective modulation of the inactivating component of current may be important in enhancing of K conductance for the expression of this form of plasticity. Unlike FMRFamide, nifedipine does not inhibit transmitter release. Taken together, the data are consistent with the idea that the two components of current only the inactivating component is important for transmitter release and for at least one form of synaptic plasticity.


FMRFamide increases the S K current in Aspura sensory neurons through lipoygenase metabolites of arachidonic acid (A.A.). Two mechanisms proposed to control release of A.A. through stimulation of phospholipase A, are an increase in Ca, or an increase in pH, due to Na/H exchange. Here we study possible roles for Ca, or pH, in the response to FMRFamide. FMRFamide had no effect on resting Ca (100 nM), as determined by fluorescence ratios with fura-2 in the soma or growth cone. However, FMRFamide did decrease the Ca transient in response to a Ca stimulus (3% and 10% Co, respectively). The Ca transient was suppressed (79%) with added growth cone (50%), consistent with its role in presynaptic inhibition. Next, the role of Na/H exchange was studied by replacing internal Na with N-methyl-d-glucamine (NMDG+). Na removal reduced the S current response to FMRFamide by 66% but did not affect the response to A.A. Thus, the Na-sensitivity lies at a stage leading to A.A. or Ca release. However, Na removal with BCFE showed no change in response to FMRFamide. FMRFamide also did not speed recovery from acidification following exposure to NH4. Arguing against marked stimulation of Na/H exchange the inhibition of the FMRFamide response in Na-free medium could be related to a decrease of 0.3 pH units we see upon Na removal. Thus, the FMRFamide-mediated release of A.A. does not require an increase in Ca, or pH.


We have measured calcium levels in individual presynaptic terminals of the excitatory axon to the crayfish claw opener muscle while recording excitatory junction potentials (EJPs). Frame 2 (17 μM in 100 μM KC1) was isotopically injected into the neuron with an electrode (15-20 nA for 30-60 min) into the presynaptic axon, which was then stimulated extracellularly while EJPs were recorded in proximal fibers. Terminals (5-7 μm diameter) filled with 185000 and an adjacent muscle fiber were imaged during stimulation using a cooled charge-coupled detective camera attached to an upright microscope with a 40X objective. Long-lasting (1-3 h) trains of the EJP, observed following tetanic stimulation of the excitatory axon (9 of 10 preparations with 7-10 min 20-33 Hz tetani). Presynaptic, with time constants of about 5.4 ms, the EJP, observed with the electrophysiology post-tetanic potentiation. Supported by NIH Grants NS 15114 and Bell Labs.
POTENTIATION OF SYNAPTIC TRANSMISSION AT AN IDENTIFIED SYNAPSE IN THE CEREBRAL GANGLION OF APLYSIA, S.M. Friedman. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The excitatory monosynaptic connection between A and B neurons in the cerebral ganglion of Aplysia exhibits homosynaptic potentiation following high frequency stimulation. Two A and two B neurons were impaled in ganglia bathed in 5x Ca++, 3x Mg++ SW to suppress polysynaptic pathways. One A neuron was driven at 0.004-0.01 Hz, producing a decrement of its EPSPs in the B neurons. An interpolated stimulation of the A neuron with a 2 sec 10-30 Hz train potentiated the EPSPs in both B neurons. The amplitude of the subsequent test EPSPs slowly increased to 150% of their initial (pre-decrement) levels, with the peak increase occurring 5-6 min after the high frequency train. A single 2 sec stimulation produced potentiation lasting longer than 20 min. Stimulating either B neuron or the second A neuron at 30 Hz was without significant effect on the EPSPs evoked by the first A neuron. This suggests a primarily presynaptic mechanism for the response. This work was supported by NIH grant NS20846 and RCNI grant RO3032.

CA++-CHANNELS SUFFICIENT FOR QUANTAL RELEASE FROM CHICK CHILLY GANGLION NERVE ENDS. L. Hackerl and J.B. Tuttle. Deps. of Physiol. and Neurosci., Univ. of Virginia School of Medicine, Charlottesville, Va 22908.

Recordings of Ca++-channels involved in quantal release have not been reported. However, synaptic Ca++-channel properties must include: low voltage activation, slow or no inactivation, block by Mn++ but not divalent or dihydropyridine blocking, and close proximity to the release site. To record synaptic Ca++-currents, muscle was grown on 1 mm spots of collagen and ciliary ganglion neurons added after 2-3 days in culture. This restricts neurite length and the available realm of synaptic interaction. Neurons were whole-cell patch clamped with the pipet containing 150 mM CsCl, 10mM EGTA-COOH, 1mM MgCl2 and buffered to pH 7.4. The bath had 10 mM CaCl2 and 2 mM EDTA. Quantal synaptic potentials were detected using microelectrodes placed in the muscle cells. In simultaneous recording from nerve and muscle pairs, three conditions were obtained: non-transmitting junctions with only non-synaptic Ca++-channels; low probability release evoked solely by non-synaptic Ca++-channels; one-to-one transmission with synaptic Ca++-channels that did not inactivate. These results indicate that intracellular Ca++ can trigger release independent of the Ca++-channel source. We conclude that synaptic Ca++ channels impart strong transmission to these synapses because response characteristics, density and location give a more exclusive linkage to the release mechanism. Supported by NSF BNS 87-08162 and the Am. Heart Assoc. (Va. Affiliate).

SYNAPTIC AND CELLULAR MECHANISMS OF CARBACHOL-INDUCED HIPPOCAMPAL RHYTHMIC SLOW WAVE (THETA). F.K.Y. Tse and B.A. MacVicar, Dep. of Medical Physiology, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

As recorded intracellularly from CA3 pyramidal neurons in hippocampal slices of 21-28 days old rats, Carbachol (20-60µM) induced 5-10 Hz depolarizations (theta) which occurred synchronously in CA3 neurons and increased in amplitude with membrane hyperpolarization. Carbachol-induced theta was blocked by atropine, TTX, kynurenic acid (an excitatory amino-acid blocker), or Ca++ channel blockers (Cd++, Co++, or Mn++); but iCAM was not blocked by bicuculline, 5-7AM external Ca++ (which blocks polysynaptic transmission), blockers of NMDA receptors, or removal of the dentate gyus. Activation of C kinase by a phorbol ester or inhibition of C kinase by preincubation in 8-7 neither activated theta nor blocked carbachol-induced theta. However, bath application of inhibitors of intracellular Ca++ release (TMB-8 or Ba++) blocked carbachol-induced theta. We postulate that hippocampal theta is not muscarinic enhancement of a non-NMDA homosynaptic excitation of CA3 neurons by amino-acid releasing interneurons with widespread connections in the CA3 region. Intracellular Ca++ mobilization but not C kinase activation is critical in carbachol-induced theta.
NEURAL PLASTICITY IN ADULT ANIMALS: PERIPHERAL

487.1 ONGOING ELECTRICAL ACTIVITY OF SUPERIOR CERVICAL GANGLION CELLS IN MICE OF DIFFERENT SIZE. J. B. Neely and A. Asanuma. Dept. Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110 and Bogomoletz Inst. of Physiology, Kiev, U.S.S.R.

Studies of neuronal synaptic system have demonstrated systematic differences in the form and innervation of superior cervical ganglion cells among closely related mammals that differ primarily in size (Purves et al., J. Neurosci. 6: 158-163, 1986). Ganglion cells in progressively larger mammals have more elaborate dendritic arbors and receive a greater number of preganglionic inputs. In the present study we asked how these structural and functional differences might alter the ongoing pattern of synaptic activity among individual neurons. Accordingly, we used intracellular recording to monitor ongoing postsynaptic activity from ganglion cells in anesthetized but otherwise intact mice, hamsters, rats, guineas pigs and rabbits.

The proportion of ganglion cells exhibiting postsynaptic activity during a standard period of observation (5 minutes) under urethane anesthesia was least in a small mammal like the mouse (30%), intermediate in animals of intermediate size such as the hamster and rat (46% and 45%, respectively), and greatest in the largest of the mammals in the series, the guinea pig (89%) and rabbit (95%). We found, moreover, that the frequency of synaptic activation was also proportional to animal size. These differences in activity patterns are presumably related to the functional requirements of animals of different bulk, and raise the interesting question of whether normal activity in other parts of the mammalian nervous system also varies in parallel with animal size.

Supported by USPHS Grants NS 18629 and 11699.


Differences in the morphology of phasically and tonically active motor terminals were examined in the closer muscle of the crayfish claw. Lucifer Yellow or HRP was injected into the axon of the phasically active fast closer excitatory fiber (FCE) and the tonically active slow closer excitor (SCE). The terminals were subsequently viewed with the light microscope. The SCE terminals had a greater total number of synaptic varicosities per muscle fiber compared to the FCE terminals. These results are consistent with an earlier seral series EM examination of short lengths of terminal, which demonstrated that the SCE had more synaptic varicosities per terminal length than the FCE. This difference in synaptic varicosities appears to be activity-dependent since previous study also demonstrated that tonic in vivo stimulation of the FCE resulted in an increase in the number of varicosities per terminal length (J. Neurosci. 6: 2252). In this system, the number of synaptic varicosities is correlated with the amount of transmitter released during repetitive stimulation (J. Neurosci. 5: 459). An examination of the time-course of the activity-dependent formation of synaptic varicosities is in progress.

Supported by NSF grant BNS-8720135.


To study mechanisms regulating synaptic efficacy we expanded motor unit sizes in sartorius muscles of adult frogs by crushing the nerve and reducing the number of motor axons innervating the muscle.

Synaptic safety margins, measured as the sensitivity of nerve-evoked twitching to lowered [Ca]z, were lower in reinnervated muscles with expanded motor units than in control reinnervated muscles. Low safety margins can be partially explained by low transmitter release from nerve terminals. Intracellular recordings revealed that both spontaneous and evoked release of transmitter was lower in muscles with expanded motor units. Nerve terminal lengths did not differ between reinnervated muscles with expanded motor units and control muscles suggesting that transmitter release per unit nerve terminal length was lower in expanded motor units. These results suggest that release and synaptic efficacy are inversely related to experimentally altered peripheral field sizes of motor axons. We are currently investigating changes in release or postsynaptic properties from expanded motor units are different at short and long postoperative times. This may provide evidence for mechanisms regulating synaptic effectiveness.

Supported by MDA and NSF.


Motor unit sizes in reinnervated adult frog (Rana pipiens) sartorius muscle was surgically increased by excising half the muscle fibers and allowing all sartorius motoneurons to reinnervate the remaining fibers. At varying postoperative times (12 days to 2 years), the muscles were removed, and the level of focal polyneuronal Intervention (PI) studied using intracellular recording. At all times postoperatively, reinnervated muscles with reduced motor units had a consistently lower level of PI than reinnervated muscles with larger motor units. Since physiological characteristics of PI recorded were more accurate at the earliest stages of reinnervation (6-14 days after nerve crush), we are using histology to estimate PI at these times. Fewer nerve terminals reinnervated musles with reduced motor units is smaller.

We have also made histological observations of synaptic structure for all muscles from which intracellular recordings were made. Our findings may offer insight into the relationship between motor unit size and competition for synaptic connections. Funded by NSF and MDA.


In order to establish the extent to which adult nerve terminals engage in remodelling, nerve terminal growth in relation to postgrowth growth was measured in both growing and Identified mouse neuromuscular junctions (NMJ). Twenty NMJ's from adult (3-6 mo) and 10 from young (3-4 mo) C57BL/6 mouse pectoral muscles were observed. The nerve terminals (NT) were situated in situ with the motor end-plate of intact muscle (J. Neurosci. 7: 3185). Both NT and postsynaptic AChR's with bungarotoxin-FITC. The animal was then placed on the microscope stage where a 100 objective, 10x eyepieces, 1.4x barco, and record the image of the NMJ. Three observations were made of each NMJ: on days 0, 4, and 8 in young mice, and on days 0, 15, and 40 in adult.

The predominant form of growth at intact NMJ's consisted of NT extension followed (within 8 days) by an extension of the corresponding AChR. NT retractions were infrequent. At the mature NMJ, gross structure did not change (Currie, J. Neurosci. 6: 158-163, 1987), but other changes occurred. Approximately 50% of the AChR's exhibited extensions of both NT and AChR complexes while approximately 50% showed NT extensions (Purves H. 1987). Some of these two forms extended in addition to the NT-AChR complexes of at least one branch. Our data indicate that intact TMJ's increase in size and complexity by net growth of NT and subsequently, AChR's. The data also argue against the notion that the adult NMJ is structurally inflexible. Rather, the adult structure results from both movement of branches and a dynamic equilibrium between ongoing excitatory and retractive events. (Supported, MDA NOl644 and AD00105).

487.7 INFLUENCE OF PERIPHERAL TARGET SIZE ON NORMAL PATTERNS OF SYNAPTIC ACTIVITY IN FROG SUPERIOR CERVICAL GANGLION CELLS. J.T. Voyvodic and A. Ivanov, Washington Univ. Sch. of Med., St. Louis, Mo. 63110 and Bogomoletz Inst. of Physiology, Kiev, U.S.S.R.

The dendritic complexity of sympathetic ganglion cells can be regulated by changing the relative size of peripheral target areas (Voyvodic, Soc. Neu. Abstr., 13:574, 1987). Thus, increasing (or decreasing) the amount of target tissue available to neurons in the rat superior cervical gland increased (or decreased) the number and length of ganglion cell dendrites. Here, we examined whether changes in target size and dendritic geometry are associated with changes in synaptic activation of ganglion cells by preganglionic inputs. Specifically, the relative target size of neurons innervating the submandibular gland was increased by partially denervating the gland at birth, causing the death of many ganglion cells and an increase in size of the dendritic arbor of the remaining neurons. Patterns of tonic synaptic activity were subsequently measured in adult rats for cells innervating either control or partially denervated submandibular glands.

In control rats, only 11% of the ganglion cells innervating the gland (N=45) exhibited ongoing synaptic activity under urethane anesthesia during a 5 minute period of observation. Experimentally increasing the relative size of the target increased the number of active neurons from 11% to 42% (N=40). Moreover, these cells showed an increased frequency of synaptic activity compared to controls, with supersetions responses increasing from 0.1 to 0.3/s, and a sharp threshold, EPSPs increasing from 0.1 to 1.5/s.

Therefore, increasing the size of the dendritic arbor by increasing the relative size of the peripheral target is associated with a higher rate of ongoing synaptic activity. We suggest that the increase in ongoing synaptic activity is due to increased preganglionic convergence onto the more complex ganglion cells produced by target enlargement.

This work was supported by NSF grants NS 11699 and NS 18629 to D. Purves.
NEURAL PLASTICITY IN ADULT ANIMALS: PERIPHERAL

487.7

NEUROMUSCULAR JUNCTIONS SHRINK AND EXPAND AS MUSCLE FIBERS CHANGE SIZE: CROSS-SECTIONAL AREA MODULATES ENDPLATE NUMBER


After the first postnatal month, neuromuscular junctions in the mouse sternomastoid are not substantially remodeled, but rather enlarge by simple elongation of existing nerve terminals and matrix (Ballman and Lichtman, in prep.). These observations are consistent with the idea that growth of an endplate is an essential feature of the development of neuromuscular junctions. The endplate is composed of a Schwann cell and a group of axon terminals that innervate a muscle fiber. The endplate is surrounded by a basal lamina, which is continuous with the basal lamina of the muscle fiber. The endplate is also associated with other structures, such as collagen fibers and extracellular matrix molecules.

DISCREPANCIES BETWEEN HISTOLOGICAL AND IN VIVO OBSERVATIONS OF MUSCLE SIZE AND SYNAPTIC REMODELING


Until recently, synaptic remodelling at neuromuscular junctions could only be inferred from histological images representing single time points. Repeated observations of junctions in living frogs have now revealed that remodelling commonly occurs under normal conditions in adults (Soc. Neurosci. Abstr. 13, 1665; 1987). Analysis of these in vivo images suggests that some of the previously unappreciated histological results may need to be re-interpreted.

1) Histology supports the view that terminal sprouts are ephemeral. They either differentiate into mature terminals or retract. When terminal sprouts are retracted, they require a stabile, neither growing nor retracting for months. 2) Empty, cholinesterase (ChE)-stained postsynaptic folds are taken as histological signs of nerve terminal retraction. Indeed, we observe that most retracting branches leave behind ChE-stained folds. However, we also see such folds beyond the distal tips of branches that have grown into stable folds. Thus, growth can be followed by partial retraction or that postsynaptic differentiation can precede terminal growth. 3) It was anticipated that remodelling may be more pronounced at polyneuronally innervated junctions, since synapse elimination is prolonged in frogs. Over periods longer than a year, growth is indeed substantial, but multiple inputs are apparently not eliminated. Supported by NIH.

487.9

NEURON/GLIAL RELATIONSHIPS OBSERVED IN LIVING MICE.

S. M. Breedlove, R. J. Balice-Gordon, and J. W. Lichtman. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Bandulin in vivo studies of motor endplates, which are stained with fluorescent dye. This result was obtained in anesthetized but otherwise intact mice (Ballman and Lichtman, in prep.). These observations are consistent with the idea that growth of an endplate is an essential feature of the development of neuromuscular junctions. The endplate is composed of a Schwann cell and a group of axon terminals that innervate a muscle fiber. The endplate is surrounded by a basal lamina, which is continuous with the basal lamina of the muscle fiber. The endplate is also associated with other structures, such as collagen fibers and extracellular matrix molecules.

DIRECT VISUALIZATION OF SYNAPTIC REMODELING IN GASTROCNEMIUS MUSCLES OF LIVING ADULT MICE.

D.J. Wiggton. Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

Dynamic rearrangement of synaptic connections can be observed directly in muscles of living animals, although different muscles may vary considerably in the extent of NMJ remodeling (Wiggton et al., 1987). Some muscles exhibit some remodeling while others do not. For example, in the slow-twist soleus muscle, nerve terminals exhibit some remodeling over 6 months (Wiggton, 1987; Soc. Neurosci. Abstr. 13:1007). To explore the possibility that the difference between sternomastoid and soleus muscles in the extent of NMJ remodeling reflects their different physiological type, I examined NMJs in another fast-twist muscle, lateral gastrocnemius (LG). I labeled acetylcholine receptors at LG NMJs in anesthetized animals with rhodamine-labeled peanut agglutinin (PNA) and with fluorescein isothiocyanate (FITC) to label AChR clusters. In LG NMJs, fluorescent terminal but a slight increase in the matrix length. Rhodamine-labelled peanut agglutinin (PNA) specifically stains the synaptic extracellular matrix at living frog neuromuscular junctions (NMJs). To examine the remodeling of synaptic matrix and its dynamic relationship with nerve terminals identified in terminal regions at adult living frog NMJs (E. pipiens), sartorius muscles were stained with 4-di-2-AAP, a fluorescent dye for 10 minutes (13 min), followed by PNA (50ug/ml, 30 min). NMJs were observed at anemotaxidished frogs. In situ with fluorescein microscopy and videomicroscopy. Three months later I remodeled and examined the same NMJs. Remodeling, in the form of additions and deletions of branches, was evident at some NMJs but less frequently than I observed in soleus muscles monitored over the same period of time. The extent of remodeling at mammalian NMJs might thus depend on the frequency of their activation.

487.11

DYNAMIC RELATIONSHIP BETWEEN SYNAPTIC EXTRACELLULAR MATRIX AND MOTOR NERVE TERMINALS IN LIVING FROGS.


Rhodamine-labelled peanut agglutinin (PNA) specifically stains the synaptic extracellular matrix at living frog neuromuscular junctions (NMJs). To examine the remodeling of synaptic matrix and its dynamic relationship with nerve terminals identified in terminal regions at adult living frog NMJs (E. pipiens), sartorius muscles were stained with 4-di-2-AAP, a fluorescent dye for 10 minutes (13 min), followed by PNA (50ug/ml, 30 min). NMJs were observed at anemotaxidished frogs. In situ with fluorescein microscopy and videomicroscopy. Three months later I remodeled and examined the same NMJs. Remodeling, in the form of additions and deletions of branches, was evident at some NMJs but less frequently than I observed in soleus muscles monitored over the same period of time. The extent of remodeling at mammalian NMJs might thus depend on the frequency of their activation.

487.12

DYNAMIC RELATIONSHIP BETWEEN SYNAPTIC EXTRACELLULAR MATRIX AND MOTOR NERVE TERMINALS IN LIVING FROGS.


Rhodamine-labelled peanut agglutinin (PNA) specifically stains the synaptic extracellular matrix at living frog neuromuscular junctions (NMJs). To examine the remodeling of synaptic matrix and its dynamic relationship with nerve terminals identified in terminal regions at adult living frog NMJs (E. pipiens), sartorius muscles were stained with 4-di-2-AAP, a fluorescent dye for 10 minutes (13 min), followed by PNA (50ug/ml, 30 min). NMJs were observed at anemotaxidished frogs. In situ with fluorescein microscopy and videomicroscopy. Three months later I remodeled and examined the same NMJs. Remodeling, in the form of additions and deletions of branches, was evident at some NMJs but less frequently than I observed in soleus muscles monitored over the same period of time. The extent of remodeling at mammalian NMJs might thus depend on the frequency of their activation.
CEREBRAL METABOLISM AND BLOOD FLOW IV


To99m-ethyl cysteine dimer (ECD) is a neutral, lipophilic complex which rapidly crosses the blood brain barrier. It has been evaluated clinically as a marker of regional cerebral perfusion. Multiplexes pharmacological evaluation of To99m-ECD show the compound to be efficacious as a brain perfusion agent only in primates. The brain pharmacokinetics of To99m-ECD are similar in humans and in non-human primates. Rapid brain uptake (initial 5% injected dose) was observed in both species, as was prolonged retention in a cerebral perfusion pattern (Neurology 38:363,1988). Results in humans and nonhuman primates suggest that the brain retention and extensive clearance of To99m-ECD are due to its rapid metabolism via ester hydrolysis. Subcellular distribution studies in monkey brain one hour post To99m-ECD administration demonstrate that more than 70% of the activity is localized in the cytosolic fraction and is primarily in the form of single polar metabolites. When this metabolite was injected into a monkey it failed to cross the blood brain barrier. These results support the hypothesis that To99m-ECD is metabolized rapidly in the brain of primates by a specific enzymatic pathway to a polar complex which is trapped.

486.3 STATE-DEPENDENT DIFFERENCES IN CEREBRAL BLOOD FLOW DURING CORTICAL SPREAD DEPRESSION IN RATS. R. B. Dworkin. Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

Cortical spreading depression (SD) is a reversible phenomenon that may occur during complicated and classic migraine headache. In anesthetized rats regional cerebral blood flow (rCBF) increases dramatically as SD advances through the neocortex. However, hyperemia has not been measured in the cortex of awake humans during migraines. The hypothesis that these flow differences are state-dependent was tested by measuring rCBF in awake and anesthetized rats during SD. Using pentobarbital anesthesia and bipolar electrodes placed on the lateral convexity of the left hemisphere and secured with dental acrylic, two days later rats were prepared under halothane/nitrous oxide anesthesia, restrained with a plaster hip-cast and allowed a one hour recovery period. SD was induced by a 5 mA direct current lasting 5 seconds and confirmed by recording electrocortical activity. Two minutes later rCBF was measured in the left hemisphere of SD and control animals by measuring the rate of clearance of a bolus of 14C-iodoantipyrine in regions isolated by gross dissection. In rats reanesthetized with pentobarbital, rCBF in the hemisphere with SD increased by 40%. In awake rats rCBF decreased by 30% in the SD hemisphere and increased by 50% in the contralateral hemisphere. These state-dependent differences in rCBF during SD are consistent with the presence of an intrinsic neural system regulating blood flow in the brain. (Supported by PHS N024109 and an ANA Established Investigator Award)

486.5 LOCAL SYMPATHETIC CONTROL OF THE CEREBRAL CIRCULATION: AN AUTORADIOGRAPHIC STUDY. U. J. Schaller, A. G.矢 LE, H. Divan. Division of Biomedical Research, Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8

The present study examined the effect of sympathetic stimulation on the local cerebral circulation. Changes in local cerebral blood flow were measured in anesthetized rats during unilateral stimulation of the superior cervical ganglion using 14C-labeled antipyrine. Two amperages were chosen on the basis of preliminary studies in 10 rats, which demonstrated that stimulation of the ganglion at 15 Hz, 3 mae and 10 V produced a 1-2 mm Hg increase in arterial blood pressure in each animal, indicating an overall reduction in cerebral blood volume and/or flow. In sham control animals (n=4), changes in local cerebral blood flow using 14C-labeled antipyrine were less than 2% in all regions examined. During unilateral stimulation of the sympathetic ganglion (n=5), blood flow in temporal muscle was increased by 140±20%. In both hemispheres there were less marked reductions in local cerebral blood flow. For example, blood flow in the cerebellar cortex was similar in both hemispheres. Blood flow in the parietal cortex and the caudate nucleus were reduced ipsilaterally to the stimulation by 9.3±2.8% and 11.0±5.4%, respectively. Reductions in flow were unevenly distributed within these regions possibly corresponding to a differential regional density of sympathetic innervation of the cerebrovasculature. Further experiments and analysis are required to map in detail the influence of sympathetic stimulation on the cerebral circulation.


We sought to determine: 1) Does electrical stimulation of the BF alter cortical CBF? 2) If so, are cholinergic receptors involved? Rats were anesthetized (chloralose), paralyzed, artificially ventilated and arterial blood gases controlled. A cranotomy was performed over the parietal cortex and microvascular perfusion, an index of CBF, measured continuously. A bipolar concentric electrode elicited remarkable increases in cortical CBF (up to 250% of resting CBF) that were frequency (2.5-100 Hz) and intensity (25-150 µA) dependent; but independent of heart rate or arterial pressure (N=7). A nicotinic antagonist, mecamylamine (4 mg/kg, i.v., 20 min prior) significantly reduced increases elicited by 52±8% (p<0.05; N=4). In contrast, the response was enhanced by 40±1% (p<0.05; N=3) with a cholinesterase inhibitor, physostigmine (50 µg/kg, i.v. In these experiments, the muscarinic antagonist, atropine (1 mg/kg, i.v.) also enhanced the response by 25%. CONCLUSIONS: 1) Cortical CBF is profoundly increased by electrical stimulation of neurons originating in or passing through the BF; 2) cholinergic neurons, possibly those in the BF, participate in this response; and 3) nicotinic receptors facilitate, while muscarinic receptors inhibit this dilator pathway (Supported by American Health Assistance Foundation).


The extent and amount of blood flow and tissue perfusion in neural grafts is an important factor in the evaluation of new tissue for the treatment of Parkinson's and Alzheimer's disease and for cerebral ischemia. Nine Sprague Dawley rats weighing 180-200 g were prepared with a 2x3 mm cavity for transplantation. Two weeks later, embryonic septal tissue from 15-16 day old embryos was placed in five of these animals. Four were not grafted. All surgical procedures were performed under halothane/6% nitrous oxide anesthesia (i.p.). Five months after transplantation (body weight ± SEM = 480 ± 8 g), cerebral (and graft) blood flow (CBF) was determined using quantitative autoradiography (Sakurada et al., Am. J. Physiol. 234H159, 1978) under physiologically controlled and stable conditions of mean arterial blood pressure (83 ± 4 mmHg), PaCO2 (34.8 ± 0.7 mmHg), PaO2 (170 ± 6 mmHg), and pH (7.422 ± 0.012). Blood flow was determined from histologic sections using polarized light. The degree of regional perfusion matched that of host tissue in Nissl stained sections.

CBF in non-grafted cortex was decreased 6% in all regions except for one animal. Grafts had normal CBFs in adjacent-to-graft cortex. Grafted animals had increased perfusion to the non-grafted cortex. Thus, there was an increase in perfusion to normal tissue. The treatment of Parkinson's disease with dopamine replacement therapy will benefit from these studies as the direct pattern of brain perfusion in the grafted area resembles that of the host. Grafted neurons exhibit clinical improvements and increased blood flow in the transplanted areas. We conclude that transplantation provides evidence for the development of normal blood flow.
INTERACTIONS BETWEEN NEUROTRANSMITTERS III

488.7 CEREBRAL METABOLISM AND BLOOD FLOW IV


The chronic administration of clinically effective antipsychotic drugs increases the concentration of neurotransin (NT) in the mid-thoracic region of the thoracic spinal cord and the nucleus accumbens of the rat brain. Since neuroleptic drugs block the dopamine (DA) receptors of mesencephalic DA neurons, we hypothesized that the presynaptic DA neurons were essential for this neuroleptic-induced increase in NT. Accordingly, adult male Sprague-Dawley rats were pretreated with desmethylimipramine (10 mg/kg, IP) or 1.2% tartaric acid vehicle for seven days and results in a greater than 90% depletion of DA in the brain after the last 6-OHDA injection. Some animals from both groups were injected daily with either haloperidol (1 mg/kg, IP) or 0.1% tartaric acid vehicle for three weeks. Rats were decapitated and the brain was used for radioligand binding assay of NT: frontal cortex, olfactory tubercles, nucleus accumbens, septum, pre-optic/diagonal band, hypothalamus, amygdala, hippocampus, ventral tegmental area (VTA), and substantia nigra. Both the 6-OHDA-treated group and vehicle controls receiving saline (p<0.01) showed significantly higher specific binding of the radioligand to the DA neuron that did not receive haloperidol. Thus, while the post-synaptic dopaminergic pathways are needed for the neuroleptic-induced increase in NT concentrations in these regions, the dopaminergic synapses are not.

Supported by NIMH NS 35513 and a grant from the Schizophrenia Research Foundation.

488.8 TOTAL IR PARTIAL BRAIN ISCHEMIA; A NEW RAT MODEL. J.C. de la Torre, J. Porter, and J. Shah. Univ. of Ottawa Health Sciences, Ottawa, Ont.

Experimental stroke models using the rat have been developed to produce partial or total arrest of blood flow to brain but no model offers both options. The four- vessel total ischemia model and several modifications of it, hinges on the fact that rats remain symptomless if fewer than 3 vessels to the brain are occluded (e.g., both vertebral arteries). This model was difficult to reproduce because vertebral artery electrolytically through the alar foramina open in brain tissue damage or undesirable bleeding despite direct visualization of the vessel. The present model was developed because the rats offer many advantages over other animal models. Rat is anesthetized with diazepam-somatol and xylocaine is sprayed orally to reduce gag reflex. Rat is intubated with a PE 100 catheter and ventilated mechanically. After a mid-cerebral incision, a hemostat is used to clamp the left post-temporal attachments from just above the bregma to just below the first rib. An electrocoagulating incision is made between sphenoid and hemostat to expose the brain. The aortic arch and great vessels are identified and the left and right subclavian arteries are occluded with microvascular clips. The chest wall is closed with sutures and 2 ligatures are looped around both common carotid arteries then threaded internally via catheters. After rat fully recovers, one or both carotid ligatures are pulled and released for varying periods of time. This procedure results in either partial or total cerebral ischemia and produces hemorrhagic damage. MRI studies show that this 3 or 4 vessel occlusion model can be easily and reliably set up to study morphologic, physiologic and chemical brain pathology under moderate or severe circulatory parameters.

Supported by the Ontario Heart Foundation.


Endothelin dependent relaxing factors (EDRF) are agents of undetermined chemical identity, produced by normol endothelium, which effect vasodilation in response to certain agonists. Recently, it has been shown that feline pal microvessels produce an EDRF when exposed to topically-applied Ach and that such a response is abolished immediately following experimental fluid percussion brain injury. Whether the post-traumatic impairment of endothelial dependent relaxation (EDR) is reversible over time, has not been determined and was examined in the present study. Asceptically, anesthetized cats were each equipped with a cranial window and subjected to a modern level of brain injury. Tail arteriole following Ach exposure was assessed prior to injury and at 30 minutes post-injury, 4, 8 and 12 hours post-injury. At each assessment, vascular diameters were measured before and after Ach application. At 30 minutes post-injury, normal Ach-Induced EDR was converted to vasoconstriction, most notably in small caliber vessels (<0.04 mm). At 4 hours, recovery of EDR was observed in a number of vessels, and by 12 hours the majority of vessels exhibited normal response, although some remained dysfunctional. Small arteries recovered sooner than large ones. The findings suggest that, in the absence of secondary insults, mechanisms underlying early posttraumatic loss of cerebral EDR do not persist and do not, for the most part induce irreversible changes which prevent recovery of EDR. These vessels, which fail to demonstrate EDR at 12 hours post-injury, may require more time for recovery or may be irreversibly damaged.

Supported by NIH Grant NS 12587

High densities of neuropeptide Y-cholceystokinin and dopamine-containing perikarya were found in the ventral tegmental area (VTA). Coexistence of these peptides in the fibers of the mesolimbic pathways originating from the VTA and projecting to the nucleus accumbens suggested a neuromodulation role in dopamine transmission by these peptides. The aim of the present study was to estimate the level of concentration of the extracellular metabolites of dopamine, i.e. DOPAC and HVA, and serotonin, i.e. 5-HIAA, in the nucleus accumbens after microinjections of neuropeptide Y or cholecystokinin (10 pmol and 10 pmol) into the VTA. The concentration of these metabolites was estimated using high pressure liquid chromatography with electrochemical detection. Our results show that the injection of distilled water and the low dosage (10 pmol) of neuropeptide Y or cholecystokinin remain without effect. The injection of saline gave rise to transient increase in Dopac while 10 pmol of both peptides induced a larger and longer increase in the extracellular Dopac and HVA while 5-HIAA never changed from the baseline level, in the nucleus accumbens after their VTA microinjections.


We have previously reported that the amphetamine (AP)-stimulated release of [3H]DA from slices of rat hippocampus can be inhibited in a dose-dependent manner by opioid agonists which act through the mu receptor, but not by those acting through delta- or kappa receptors. In contrast, K+-stimulated release of [3H]DA from striatal slices can be inhibited by kappa opioid agonists, but not by mu or delta agonists. We now report that release of these catecholamines can be stimulated by glutamate and N-methyl-D-aspartate (NMDA) in a dose-dependent manner, quisqualate and kainate were much less effective in stimulating release. Release stimulated by 100 μM NMDA could be blocked by the antagonist 2-amino-5-phosphono-valeric acid (APV, 50 μM) or by the inclusion of [3H]DA in the incubation medium. Consistent with the pharmacological profile for inhibition of K+-stimulated release, the mu-opioid agonist Tyr-D-Ala-Glu-N-Methyl-D-aspartate (DADLE) inhibited NMDA-stimulated release of [3H]DA release from striatal slices, while the kappa agonist U-50,488H inhibited NMDA-stimulated [3H]DA release from striatal slices over concentration range equivalent to those required for inhibition of K+-stimulated release. Taken together, these data suggest that amino acids which interact with NMDA receptors, and opioids, acting through mu or kappa receptors, may participate in regular regulation of catecholaminergic pathways in rat brain.
FRIDAY AM

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

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CENTRAL CHOLINERGIC INNERVATION OF THE DOPAMINERGIC AND SEROTONERGIC
SYSTEMS. B.K. Hartman, P.L. Faris* S.J. Kalmbach*1, C. Cozzari*2, A.
Berod*3 . Dept. Psychiatry, Univ. of Minn, Mpls. MN 55455, Wash. U. Sch.
Double label immunohistochemistry was used for the simultaneous
visualization of choline acetyltrans ferase (CAT) and tyrosine hydroxylase
(TH) or serotonin (5-HT) to investigate the interactions between the pon­
tomesencephalic cholinergic group of neurons (PMCG) and the dopamine and
5HT systems in rat brain. First, CAT was localized using the PAP tech­
nique with nickel-cobalt enhanced DAB. Sections were then processed for
either TH or 5-HT localization using PAP with alpha-napthol as substrate
and differentiated with the dye pyronin-B. The jet-black CAT positive
cells and processes contrasted well with the bright magenta staining of
the TH or 5-HT positive structures, making evaluation of interactions
feasible at the light microscopic level.
The ventral-rostral part of the PMCG extended to the caudal limit of
the dopaminergic neurons of the substantia nigra. CAT-positive axon
fibers were observed to enter the nigra where they formed numerous boutonlike contacts with TH-positive dendrites and soma. Interactions were
restricted to the caudal half of the nigra and the ventral tegmental dopa­
mine neurons. The dorsal part of the PMCG was observed to send axon
fibers medially and rostrally into the dorsal raphe where a small number
of interactions with 5-HT-positive neurons occured. CAT-positive axons
then turned ventrally to enter the midline raphe, again making contact.
On the basis of these results and previously reported interactions bet­
ween this cholinergic group and the noradrenergic neurons, it is hypothe­
sized that an important function of the PMCG is to coordinate the
functions of the three biogenic amines. Supported by NS-12311 (BKH), RSDA
MH-00595 (PLF).

EVIDENCE FOR A FUNCTIONAL INTERACTION BETWEEN THE SEROTONIN (5-HT)
REUPTAKE
PROCESS
AND
α 2-ADRENERGIC
RECEPTORS
LOCATED
ON
5-HT
TERMINALS IN THE RAT HYPOTHALAMUS. P. Blier. A.H. Galzin* and S.Z.
Lanqer. Laboratoires d 'Etudes et de Recherches Synthélabo (L.E .R .S.),
58, rue de la Glacière, 75013 Paris, France
The efficacy of presynaptic receptor agonists to inhibit the
electrically-evoked release of [3H]-monoamines from preloaded brain
slices was shown to be attenuated in the presence of reuptake blockade
for the 5-HT and the noradrenaline (NA) systems. There is controversy,
however, as to the involvement of a functional link between the presynaptic receptors and the reuptake carriers or of a competition between
the exogenous agonist and the neurotransmitter for the receptor sites.
In order to verify the concept that such a functional interaction could
exist, we undertook to study the α 2 -adrenergic-mediated inhibition
of electrically-evoked [3H]-5-HT release from preloaded slices of the
rat hypothalamus, a model in which endogenous NA does not reach the NA
heteroreceptors located on 5-HT terminals. Two periods of electrical
stimulation were applied 60 min (S1) and 104 min (S2) after the
onset of superfusion. Drugs were added 20 min before S 1 or s2 . The
NA
reuptake
blocker desipramine
(0.3 μM)
did not alter
the
electrically-evoked release of [3H]-5-HT or the inhibition produced
by UK 14.304 (0.001-10 μM), an α 2 -adrenergic agonist. The 5-HT
reuptake blockers citalopram (0.01-1 uM) and paroxetine (1 pH),
which by themsąlves did not modify [3H ]-5-HT release, decreased the
inhibition of [3 H ]-5-HT release produced by UK 14.304. The effect of
exogenous NA (0.1-1 pH) on [3h ]-5-HT release was also attenuated in
the presence of citalopram. In contrast, citalopram modified neither
the electrically-evoked release of [3h ]-NA nor the UK 14.304-mediated
inhibition of [3h ]-NA release. The interaction was also present in
slices obtained from rats with depleted endogenous stores of 5-HT.
Activation of either protein kinase C or of adenylate cyclase together
with phosphodiesterase inhibition, which attenuate the UK 14.304inhibition of [3H]-5-HT release, did not hamper the interaction
between UK 14.304 and citalopram. In conclusion, the presynaptic NA
heteroreceptor and the 5-HT reuptake carrier appear to be functionally
linked. This interaction cannot be attributed to an increased synaptic
availability of either NA or 5-HT or to an activation of either the
phosphatidyl inositol cycle or the adenylate cyclase system.

489.11

489.12

DEVELOPMENT OF DEPOLARIZATION INDUCED GLUTAMATE RELEASE AND ITS MODULATION BY

THE EFFECT OF TRYPTOPHAN HYDROXYLASE INHIBITION ON
SUBSTANCE P mRNA DEVELOPMENT IN THE MEDULLARY RAPHE
NUCLEI. P.D. Walker. L. Ni*. T.L. Green*. S. Schotland*. R.P. Hart, and
Substance P is co-localized with serotonin (5-HT) in almost all o f the neu­
rons developing in medullary raphe nuclei, B1 and B2 (Ni and Jonakait, Soc.
Factors regulating co-localized neurotransmitter
molecules during development are ill-defined. We sought to determine possible
changes in SP mRNA levels following inhibition of tryptophan hydroxylase
(TPH), the rate-limiting enzyme in 5-HT biosynthesis.
Pregnant rats received Alzet minipump implants containing p-chlorophenylalanine (pCPA) on day 8 of gestation (E8). Each pump contained an amount of
p CPA sufficient to deliver 100 m g/kg/day for 14 days. In addition, a 300 m g/kg
s.c. injection was given at the time of implantation. Control animals received
sham surgery with no pump implantation. As measured by radioenzymatic
assay, TPH activity in dorsal raphe nuclei from pCPA-treated litters was
inhibited by 60-70% from E14 to post-natal day 3 (PND3) returning to control
levels at PND 8. Total RNA isolated from medullary raphe nuclei o f control
and p CPA-treated litters was subjected to Northern blot analysis using a
radioactive RNA probe for rat preprotachykinin (subclone from pGem 2-31-1,
kindly provided by J. Krause, Washington University, St. Louis, MO) In contrast
to TPH activity, SP mRNA levels were down to 7% of control at E14 and
steadily increased to equal control levels from E19 to birth. At PND1, SP
m RNA levels were increased four-fold and then fell to control levels by PND8.
These results suggest that inhibition of 5-HT biosynthesis has regulatory
consequences for co-localized peptide neurotransmitters. (Supported by NS
23687. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.)

SEROTONIN IN CORTICAL NEURONS.

1213

L. S ikich and R. D. Todd. (SPON: E. Robins)

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MO 63110.
Glutamate has been postulated to play roles in both normal developmental
processes such as synaptogenesis and in pathological processes such as
perinatal neuronal death from asphyxia. However, the developmental time course
of glutamate release and of its modulation by other neurotransmitters has not
been well characterized. In adults, glutamate release is elicited by depolariza
tion and is inhibited by serotonin (5HT).

In the cerebellum, depolarization

induced glutamate release can not be demonstrated until the granule cells begin
to mature at PND 14-20. Neurons in the cortex mature much earlier and might
develop glutamate release during embryonic life. However, this has not been
examined. Likewise, the development of sensitivity to 5HT has not been examined.
This work addresses both questions in a dissociated cell culture system.
Cortices from E13 to E22.5 fetal rats were dissected, triturated and plated.
The cultures were loaded with 100 nM 3H glutamate, washed extensively and
depolarized with a high K+ solution. The cultures were electrophysiologically
healthy and cells survived the experimental manipulations. Glutamate release
was minimal from E13 to E17, then increased rapidly to a peak level "two-fold
greater than baseline at E19.5, and finally fell to minimal levels by E22.5.
The effect of 5HT on glutamate release was tested by exposing cultures to 100
nM 5HT immediately before treatment with the high K+ solution.

Under these

conditions, 5HT did not influence depolarization induced glutamate release.
This work demonstrates that depolarization induced glutamate release is
present prenatally and can be studied in a culture system of living, embryonic,
cortical cells. Serotonin modulation of glutamate release was not detected.

CATECHOLAMINES VI
490.1

490.2

DOPAMINE (DA) SYSTEM RESPONSES IN THE AMYGDALA (AM) AND
CAUDATE-PUTAMEN (CP) TO ACUTE VS. REPEATED REMOXIPRIDE
TREATMENT IN THE RAT. B.C. Essig* and I.C. Kilpatrick*
(SPONs P. Dean). Dept. of Pharmacology, Medical School,
University Walk, Bristol BS8 1TD, England.
Current data suggest that an action of neuroleptic
drugs on DA systems in non-CP loci may confer their anti­
psychotic activity. Here, we report the influence of an
atypical neuroleptic, remoxipride (RMX) on DA utilisation
in AM and CP after both acute and chronic treatments.
RMX (2.1 mg/kg i.p.) or its vehicle was given to male
Wistar rats (n=10-12 per group) as either single (acute)
or repeated injections once daily for 10 days. Food and
water were freely available. Two hours after the last
dose, AM and CP were assayed for DA and its two major
metabolites, DOPAC and HVA by an HPLC-EC method.
Acutely, RMX evoked large elevations in DA utilisation
in both AM (DOPAC +59%, p<0 .001,· HVA +163%, p<0.01) and
CP (DOPAC +232%, p<0.01; HVA +236%, p<0.01). After 10
days RMX treatment, these changes were less pronounced in
CP (DOPAC +43%, p<0.001; HVA +75%, p<0.01). In the AM,
the relative increase in HVA concentration was almost
halved (+71%, p<0.05). A small elevation in DOPAC
remained (+14%) but this was no longer significant.
These data suggest that the profound neurochemical
'tolerance' of DA systems seen in CP to repeated dosing
with RMX can also be seen to a lesser degree in AM. In
view of the report that meso-amygdaloid DA neurones do
not appear to possess autoreceptors that regulate DA
synthesis (Kilts et al., J. Neurosci. 7, 3961-3975), the
present data may reflect an RMX-specific action.
We thank Astra Alab (Sweden) for supplies of RMX.

EFFECTS OF RISPERIDONE (RIS), A NEW NEUROLEPTIC, ON
NIGRAL-STRIATAL DOPAMINERGIC (DA) NEURONS. P.S. Blum and
C.B. Davis*. Department of Biological Research, Janssen,
Research Foundation, Spring House, PA 19477.
RIS was compared to haloperidol (HAL) on DA neurons
using two methods. A) apomorphine (APO)- induced inhibition
of DOPA synthesis, and B) activity of DA neurons in the
substantia nigra (SN). To measure DOPA synthesis, rats
were treated with gamma-butyrolactone to block activity of
SN neurons, and NSD-1015 to block DOPA decarboxylase.
After treatment, striatal DOPA levels were 22.4+0.9 pmol/
gm, about 10 times the levels in untreated animals (2 .8±0 .2
pmol/gm). DOPA levels were reduced 51% by APO pretreatment
(1 mg/kg i.p.). Both RIS (ED50=0 .32 mg/kg i.v.) and HAL
(ED 5o = 0 .0 6 mg/kg i.v.) blocked APO-induced inhibition of
DOPA synthesis. These data suggest that RIS, like HAL, is
a presynaptic D-2 antagonist at the terminals of DA
neurons. In separate experiments, recordings were made
from DA neurons in the SN of chloral hydrate anesthetized
rats. APO inhibited activity in these neurons (ED50 =
12±2 µg/kg i.v.), and HAL blocked this inhibition with a
threshold dose of 20 µg/kg i.v. RIS, however, did not
consistently block APO-induced inhibition of neural
activity. Rather, RIS either had no effect (to 80 µg/kg
i.v.) or produced an abrupt cessation of neural activity
(between 80 and 320 µg/kg i.v.). Unlike HAL, and unlike
the effect of RIS at the nerve terminal, RIS had no
detectable effect at D-2 receptors in the SN.


490.4

The relationship between acute administration of precursor amino acids (AA) and monamine (MA) metabolism has been well elucidated in adult rats. However, the effect of chronic AA administration on MA metabolism in altered physiological states is less well understood. Therefore, we examined the effect of AA supplementation on MA metabolism during pregnancy, 205 casein diets, enriched with 55 L-phenylalanine (PHE) or equimolar tyrosine (TYR), were fed to pregnant (PREG) rats from G1 to G20 and nonpregnant (NPREG) rats for 9 days. The MA, only dopamine (DA) metabolism was altered during pregnancy. Despite similar brain TRP levels, steady-state levels of DOPAC of DOPAC, the major metabolite of DA, were reduced during pregnancy in striatum (1080±128 vs 1118±43 ng/g; X±SEM for NPREG and PREG rats) and remaining hemispheres (110±7 vs 85±4; X±SEM) for NPREG and PREG rats). Feeding precursor AA elevated DA in PREG rats to NPREG levels. No effect of pregnancy on steady-state levels of either norepinephrine or serotonin and its metabolite 5HIAA was observed. The results indicate that DA metabolism is decreased in pregnancy, and that this effect can be alleviated by dietary AA supplementation. (NSERC).

490.5
DIFFERENTIAL REGULATION OF MELATONIN-SECRETIVE DOPAMINE NEURONS BY THE SERTONERGIC ADRENA D-ADRENLIN RECEP TORS, A. M. Eamonn, R. H. Both, and A. V. March. Deps. Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The selective stress-evoked activation of the dopamine (DA) neurons innervating the prefrontal cortex (PFC) does not appear attributable to a lack of impulse-modulating autoreceptors on the AA DA neurons which also lack these autoreceptors are not activated by acid, blood, or stress. These data suggest that stress-induced changes in DA turnover may be critical to the regulation of central DA systems. The serotonin innervation of the midbrain DA neurons may be a control system for DA. We have examined the dynamics of the serotonin agonist 8-hydroxy-2-DEA, which at low doses is an autoreceptor agonist, on both basal activity and stress-evoked activation of the mesoclopalecetal DA systems. DA turnover, as reflected by DOPAC/DA, was significantly increased in the PFC by 125 µg (but not 75 µg) 8-CH-DPAT. DA turnover was not increased in the nucleus accumbens or stria. The stress-induced activation of mesoprefrontal DA neurons was essentially unaltered by 8-CR-6. However, the high dose of 8-CR-6 resulted in a stress-induced increase in DA turnover. These data suggest that 5-HT mechanisms may differentially regulate the mesolimbic DA sub-systems.

490.6

We have studied the effects of restraint (immobilization) stress on dopamine (DA) utilization in projection areas of the mesocerebral (prefrontal cortex - PFC), mesolimbic (nucleus accumbens - NAC), and mesocerebellar (supratrigeminal) neurotransmitter systems. Female Long-Evans rats were restrained in Plexiglas cylindrical tubes for 15 minutes, 30 minutes or 60 minutes and immediately sacrificed. Bilateral samples of several brain regions were removed and assayed for DA, DOPAC and HVA content by HPLC with electrochemical detection. At 15 minutes, DOPAC/DA was increased in the PFC; 3,000 µg/ml of restraint in the striata. The data suggest that, as stress is prolonged, different DA systems, as well as different sides of the PFC, are sequentially activated. (Supported by NIDA grant DA0817 to S.D. G.).

490.7
REGIONAL AND SIDE DEPENDENT EFFECTS ON DOPAMINE UTILIZATION INDUCED BY DIFFERENT DURATIONS OF RESTRAINT STRESS. J. P. Carlson, S. D. Gluck and J. L. Baum. Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

We have studied the effects of restraint (immobilization) stress on dopamine (DA) utilization in projection areas of the mesocerebral (prefrontal cortex - PFC), mesolimbic (nucleus accumbens - NAC), and mesocerebellar (supratrigeminal) neurotransmitter systems. Female Long-Evans rats were restrained in Plexiglas cylindrical tubes for 15 minutes, 30 minutes or 60 minutes and immediately sacrificed. Bilateral samples of several brain regions were removed and assayed for DA, DOPAC and HVA content by HPLC with electrochemical detection. At 15 minutes, DOPAC/DA was increased in the PFC. At 60 minutes, restraint reduced the signal, although the left side was still higher than the right. The NAC was bilaterally elevated at 30 minutes and at 60 minutes, significantly more of the stress signal was present in the striata. The data suggest that, as stress is prolonged, different DA systems, as well as different sides of the PFC, are sequentially activated. (Supported by NIDA grant DA0817 to S.D. G.).

490.8
EFFECTS OF DOPAMINE DEPLETION ON STRESS-INDUCED CHANGES IN MESOLIMBIC AND STRIATOLIMBIC DOPAMINE FUNCTION, W. A. Clark, B. H. Roth, and A. Y. D. Stein. Deps. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

We have studied the effects of restraint (immobilization) stress on dopamine (DA) metabolism in the prefrontal cortex (PFC). More severe stress recruits other DA neurons, such as those projecting to the nucleus accumbens (NAC). It is unclear whether DA release in the PFC is a permissive event (initiator) allowing other catecholaminergic neurons to respond, or whether DA release is part of a coping response. Previous data indicate that DA autoreceptor-selective doses of apomorphine are anxiolytic and potentiate DA and DOPAC/DA release. We have therefore examined the effects of stress on DA systems in the rat NAC and striatum (CP) after 6-hydroxydopamine (6-OHDA) lesion of the PFC. A marked stress-evoked increase in DA metabolite DOPAC was observed in the NAC, but not CP. Lesions of the DA innervation of the PFC blocked the stress-induced increase in DA metabolism in the NAC, but did not significantly alter DOPAC levels in the CP. These data suggest that the preferential activation of mesoprefrontal DA neurons evoked by stress may be necessary for the subsequent involvement of other catecholaminergic neurons which contribute to means of coping with stress.
MAO-B and MAO-A as well as biochemical markers of development might be due to either parameter. We will be more sensitive to MPTP than young adults whereas Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

We will be more sensitive to MPTP than young adults whereas neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Neurotoxicity is dependent upon the monoamine oxidase-B (MAO-B) catalyzed oxidation of MPTP to a dihydropyridinium intermediate which in turn plays an important role in the toxicity to PC-12 cells. The dopaminergic neurotoxicity of MPTP is dependent upon the monoamine oxidase-B (MAO-B) catalyzed oxidation of MPTP to a dihydropyridinium intermediate which in turn is oxidized to the pyridinium species MPP+ and 2'ethyl-MPP+.


The administration of MPTP or MPP+ analog, such as 2'-CH3-MPTP, to experimental animals leads to the destruction of dopaminergic neurons of the nigrostriatal pathway. Neurotoxicity is dependent upon the monoamine oxidase catalyzed formation of pyridinium species (MPP+ or 2'-CH3-MPP+) from the tetrahydrodipyrindine, and selective uptake of the pyridinium species into dopamine (DA) neurons by the DAT transport system with inhibitors of DA uptake protect against neurotoxicity. In experiments with neostriatal slices, the pyridinium ions formed from MPTP and 2'-CH3-MPTP inhibited complex 1 of mitochondrial electron transport, and thereby increased lactate (LAC) production. DA uptake inhibitors (i.e. Win 35,428, Mazindol and McN 5908) reduced 10µM 2'-CH3-MPTP-inhibited LAC formation by 57%, 45% and 34%, respectively. Win 35,428 lowered 5µM-inhibited LAC accumulation to 50% MPTP by 45%. Correlations will be made between the capacity of uptake inhibitors to decrease the tissue content of the pyridinium species, and LAC formation. These results are consistent with the concept that toxic effects of MPTP and 2'CH3-MPTP are due to uptake of the pyridinium species into the DA neuron, and subsequent mitochondrial damage.

The dopaminergic neurotoxicity of MPTP is dependent upon the monoamine oxidase-B (MAO-B) catalyzed oxidation of MPTP to a dihydropyridinium intermediate which in turn is oxidized to the pyridinium species MPP+.

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MPTP administration causes a degeneration of the nigrostrial dopaminergic pathway in mice. In contrast, rats are considerably less sensitive. In the present study, we administered MPTP to male Sprague-Dawley rats (Charles River) and male Swiss-Webster mice (Iaconic Farms) and measured brain levels of MPTP and its major metabolite 1-methyl-4-phenylpyridinium (MPP+). In contrast to what is generally accepted, we found similar levels of MPP+ in the brains of rats and mice over a 2 hour period and a single 6-Cl-DOPA injection of 80 mg/kg of MPTP. In parallel experiments, MPTP administration caused a dopamine depletion of approximately 70% in the mouse neostriatum but had no significant effect in the rat neostriatum. These data suggest that the differential sensitivity of rats and mice to MPTP is due to more than simply the brain level of MPP+. We have devised different dosing regimens in an attempt to maintain high brain levels of MPTP and MPP+ for extended time periods. Experimental variables included: the dose of MPTP, the number of injections, and the interval between injections. By altering the above variables we have been able to obtain a substantial depletion of neostriatal DA in the rat. Reasons for the differential effects of MPTP in the rat and the mouse will be discussed.


METH or MPTP administration to mice damages nigrostrial dopaminergic neurons. Toxicity induced by METH appears to depend on factors that involve the enkephaline-Dole-Wu strain and MPTP in female CD-1 mice, a nonsensitive strain. The exact cellular mechanisms responsible for cell death by METH or MPTP are unknown, oxidative stress has been implicated for both. It has been suggested that oxidative stress associated with massive release of excitatory amino acids (EAs), such as glutamate, might underlie reperfusion or postsynaptic neurodegeneration. Reperfusion damage can be prevented by (+)-MK-801, a non-competitive antagonist of the N-methyl-D-aspartate receptor. In the present study, we found that (+)-MK-801 prevented METH-induced, but not MPTP-induced, dopaminergic neurotoxicity. In mice treated with METH, neostriatal DA content and tyrosine hydroxylase activity were approximately 50% of control values; administration of (+)-MK-801 prior to and after METH prevented these decrements. These results suggest that oxidative stress mediated by EAs may play a role in the neurotoxic actions of METH, but not those of MPTP. These observations may have implications for several neurodegenerative disorders where oxidative stress might be involved in pathogenesis of the disease.

491.1 MPP+ IS NEUROTOXIC TO CEREBELLAR GRANULE CELLS. A. Martin, T. S. Nowak, J. K. M. Newrock, CNB and LNHS, NIH, Bethesda, MD 20892.

We have developed a neuronal culture system to study the neurotoxicity of MPP+. Cerebellar granule cells are susceptible to the neurotoxic effects of MPP+ at 50-100uM, evident as a loss of neuronal processes and disintegration within several days. In contrast, MPP+ is not toxic. In cocultures with sympathetic astrocytes, MPP+ is selectively neurotoxic to the granule cells, and this toxicity is prevented by the addition of the MAO inhibitor pargyline. Preliminary results in pure granule cell cultures indicate that MPP+ at 30 uM or more results in a striking reduction of phosphocreatine (Pcr) in the absence of significant ATP loss. The data suggest that these culture systems are suitable for studying several aspects of the neurotoxic effects of MPTP and MPP+, including components of uptake and metabolic conversion.


Neuronal cells seem to be involved in toxic mechanism of the potent drug N-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). MPTP toxicity is probably mediated by its toxic metabolite MPP+. Astrocytes, which contain high levels of MAO-B, seem to be involved in the in vitro study directly showed that pure cultures of mammalian astrocytes are capable of converting MPTP to MPP+. In order to better understand the glial cells role in promoting neuronal degeneration we studied the correlation between the metabolic properties of cultured rat cerebellum astrocytes and the survival of cultured PC12 cells considered as a model of dopaminergic neurons. We exposed astrocytes to different MPP+ concentrations and evaluated cell viability and collected the conditioned media at several incubation time. Preliminary data showed the conditioned medium of MPTP-treated astrocytes seems to be more toxic on PC12 cells than non-conditioned medium containing similar MPP+ concentrations. We discuss these data in terms of astrocytes involvement in MPTP neurotoxicity.


1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a catecholaminergic (CA) neurotoxin in man, mouse, and several rat and mouse strains. Diethyldithiocarbamic acid (DDC) increases CA depletion and the glial response in C57Bl/6 mice. In parallel, we report memory (500 mg/kg i.p.) and MPTP (5, 10 or 20 mg/kg i.p. X 2 every 2 h) in female CD-1 mice, a nonsensitive strain. To be more toxic, MPTP must be metabolized to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium (MPP+). To partially eliminate kinetic and metabolic factors, we studied the effect of DDC on ICV MPP+ (10 or 20 uM) and on bovine adrenal medullary (BAM) cells in culture (0.3 mM MPP+ and 0.3 - 3.0 mM DDC) was also examined. DDC increased the general toxicity of MPTP and increased CA depletions at both 5 and 10 mg/kg MPTP. DDC also increased MPP+ toxicity in neurons as evidenced by decreased CA levels and increased total protein. ICV MPP+ toxicity was also increased. It appears DDC can enhance the neurotoxicity of MPTP and MPP+ in nonsensitive mouse strains.
NEUROTOXICITY IV

Twenty days after the last dose the retinae were incubated with anti-tegmental area of 8-10 week old C57 black mice (Senluk and Tatton, Neuroscience Unit, The Toronto Hospital and Department of Physiology, supported by AH activity (ID 50 for buf 1x 10^-4 mg/kg and ID 50 for AH 5 mg/kg)) given intraperitoneal MPTP according to two schedules: 30 mg/kg/day for 10 days (300 mg/kg total) or 30 mg/kg twice in one day (60 mg/kg total). In vitro studies were also carried out.

Supported in part by a grant from Johns Hopkins C.A.A.T.

20,3 cells/mm^2. The AM somas and terminal locations corresponded to those previously described for rabbit retinae.

The effects of a two-fold increase in dietary VE (from 35 to 70 IU/1lb of dry food) for 12 weeks were examined on the dopaminergic neurons. MPTP-resistant mutants resulting from retroviral insertion can be used to identify the genes involved in MPP" neurotoxicity.

We have determined independently 1) frequency of infection (1x10^-5), and 2) frequency of spontaneous mutations resulting in MPP" resistance (1.4x10^-4). The number of virally infected MPP" resistant colonies we obtained (1x10^-5) is approximately 100 times greater than the number predicted if these events (viral infection and MPP" resistance) are unrelated. This shows a significant correlation between viral interjection within the selected mutants and induction of a mutation resulting in resistance to MPP".

We have obtained a large number of MPP" resistant clones. Analysis of the proviral positions within these mutants will allow us to identity and characterize the gene targets involved in MPP" neurotoxicity.

1. MPTP is a neurotoxin which causes an irreversible Parkinson's disease-like condition also indistinguishable from idiopathic Parkinson's disease. It can be detoxified by metabolism via the cytochrome P-450 enzyme system (Weissman et al., J Med Chem, 28:997-1001, 1985). Bufarol hydroxylase is thought to be the cytochrome P-450 enzyme capable of detoxifying MPTP in the brain (Ferreira et al., Biochem. Biophys. Res. Commun, 148: 1144-1150, 1985).

We have studied the in vivo effects of MPTP on buf and aryhydrocarbon hydroxylase (AH) activities in mice. In mouse brain, we found that MPTP (given IP) inhibited buf and AH activities in a dose-dependent manner. MPTP appears to selectively depress buf activity and is about 2300 times more in depressing buf activity compared to AH activity (ID 50 for buf 1x10^-4 mg/kg and ID 50 for AH 2.3x10^-4 mg/kg). In vitro studies were also carried out.

The possible clinical implications of our findings with respect to Parkinson's disease will be discussed.

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Supported in part by a grant from Johns Hopkins C.A.A.T.
Temporal Changes in Dopamine in Rats with MPTP Induced Motor Deficits: D. M. Wahl, M. G. B. Nobile, and M. L. Luh, Dept. of Physical Therapy Education and Dept. of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

We have examined the locomotor activity and gait patterns following treatment with MPTP in rats. The alterations in gait included a shortening of the stride, a lack of smoothness, and an increased tendency to walk with a flat-footed gait. The purpose of this project was to investigate the changes in dopamine (DA), noradrenalin (NA), and serotonin (5HT) concentrations in frontal cortex, striatum and substantia nigra 30, 60 and 90 days after injection of MPTP or solvent. Year-old rats were injected with MPTP or solvent (20 mg/kg, ip) for 7 days. Brains were removed 30, 60 or 90 days after the last MPTP injection and quickly frozen. Subsequently the brains were regionally dissected and prepared for chemical analysis using HPLC with electrochemical detection. At 30 days post-injection DA levels in the striatum were 45% of control; at 60 days, 65% of control and at 90 days 72% of control. DA levels in the substantia nigra were not significantly reduced at any of the time periods investigated. These data indicate that there is some recovery of DA levels in the striatum with time following chronic administration of MPTP in the rat. Recovery of gait abnormalities lag behind the recovery of DA levels and presently evaluating the extent of gait abnormality with DA levels to determine the correlation between the behavior and neurochemical changes. (NSHS Grant HS 21214)

The Effect of MPTP on Brain Cholecystokinin Concentration in the Mouse: G.A. Delacourt, W.A. Ransohoff, S. Sandler, and A. Magistro (SPON: G. Lehrer). Veterans Administration Medical Center, Bronx NY and Center for Neurochemical Diseases, University of Pennsylvania.

Cholecystokinin octapeptide (CCK-8) and dopamine (DA) have been shown to colocalize in meso-limbic neurons. To determine the effect of MPTP (DA neurotoxin) on brain CCK-8 and DA levels in aged mice, mice were subcutaneously injected with MPTP (30 mg/kg at time 0 and 6 hrs) or vehicle, then on day 5 sacrificed. Striatum (S) and olfactory tubercle (OT) were dissected and stored at -50°C until extraction and assay (radioimmunassay for CCK-8 and HPLC with electrochemical detection for DA). Results (mean±SEM):

<table>
<thead>
<tr>
<th>Section</th>
<th>CCK-8</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control OT</td>
<td>1.24±0.1</td>
<td>11.3±0.9</td>
</tr>
<tr>
<td>Control S</td>
<td>1.02±0.1</td>
<td>15.3±1.6</td>
</tr>
<tr>
<td>MPTP OT</td>
<td>0.86±0.1</td>
<td>11.0±0.2</td>
</tr>
<tr>
<td>MPTP S</td>
<td>0.90±0.1</td>
<td>11.2±0.1</td>
</tr>
</tbody>
</table>

All concentrations: ng/mg protein. HVA- homovanillic acid. DOPAC-dihydroxyphenylacetic acid. P<0.001. Conclusions: While MPTP markedly reduced DA concentration in S and OT (2) increased DOPAC/HVA/DA in S suggest a compensatory up regulation of surviving DA terminals (3) MPTP does not decrease CCK-8 concentration in S and OT, possibly because of neuronal differences in uptake and/or metabolism of this neurotransmitter.
492.3
CEREBELLAR BLOOD-BRAIN BARRIER (BBB) IS DAMAGED BY INGESTION OF ASACRINE IN POLYIC SODIUM. J. Neiman*, P.A. Stewart, C.R. Farrell*, P.L. Carlin*, J. Orrego*, Addiction Research Foundation, 33 Russell St., Toronto, Ontario, and Departments of Anatomy and Medicine, University of Toronto, Toronto, Ontario, Canada, MSS 1AB.

Cerebellar damage and liver disease are frequently seen in alcoholic patients with hepatic encephalopathy. It can become coma-toxic after ingesting high levels of protein. The pathogenesis of these effects probably includes changes in the composition of the extracellular fluid. We postulate that blood-brain barrier breakdown plays a role in these phenomena. Hepatic cirrhosis was produced in rats by ligating the bile duct. The lesion of the liver was 2-4 weeks, half of the sham-operated rats were force-fed 15 g/kg casein hydrolysate. The effect of feeding the operated rats was surprising, and not all sham-operated rats were force-fed. Thus, the BBB was evaluated using an intracerebral injection of horseradish peroxidase (HRP). 30 seconds after injection, the rats were decapitated, and the brains removed and examined for HRP leakage. Within the cerebral cortex the BBB appeared to be intact in all animals, however in animals with both bile duct ligation and multiple, large leakage spots were found in the cerebellum, primarily in white matter. The BBB damage in the cerebellum suggests that liver disease and dietary proteins may play a role in the cerebellar damage seen in alcoholic patients.

492.5

Glutathione (GSH) plays an important role in the protective mechanisms of cells. This study evaluated the effects of buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, on GSH levels in the striatum, substantia nigra and locus coeruleus in young and aged mice. All animals were HPLC operated. GSH values in the striatum, substantia nigra and locus coeruleus of the young control animal were 2.14, 1.47 and 3.12 nmol/kg respectively. BSO induced a decrease in GSH levels in animals injected with 1.5 to 6.0 mM concentrations of the drug. GSH was significantly reduced in the three areas within 5 hours following repetitive injections of 4.5 mM BSO for 24 hours. However, a return to control values occurred in all tissues after 72 hours. Comparison between young and old controls showed that a 15% decrease occurred in GSH concentrations in the striatum and substantia nigra, while the difference between young and old animals in the locus coeruleus was over 40%. The effect on GSH levels of old mice injected with 3.0 mM BSO was comparable to the effect observed in young animals. The decreases in GSH concentrations in the young animals from the same drug exposure is presently unknown whether the further decrement in GSH levels from BSO treatment in aged mice has a critical effect on catecholamine neurotransmitter systems. Supported by PHS grants AG00300, NS16147, and United Parkinson's Foundation.

492.7
AXONAL PROTEIN TRANSPORT DEFICIENCIES FOLLOWING CHRONIC EXPOSURE TO ACRYLAMIDE (ACR) AND 2,5-HEDAMINE (HEM). D.W. Slocum* (SPON: G. Standa), Department of Anatomy, Medical College of Georgia, Augusta GA. 30912-2000.

Single injections of acrylamide (ACR) and 2,5-hedamine decrease the rate and quantity of protein transport in rat sciatic nerve (Slocum, Toxicologist 8:43, 1988). These studies (segmental analysis of somatic nerve radioactivity after 3H-leucine DRG injection) have been extended to determine the overall effect of the neuropathy-producing intoxication schedule upon protein transport to the axon. Over a 24-hour period following a single injection of 50 mg/kg ACR and 4mMoles/kg HEM, the rate was equally reduced 6.1% and 8.7%, respectively, in comparison to control. The rate of ACR or HEM produced a flat 7.8% and 13.1% reduction, respectively. The quantity of protein transported was decreased 54% following a single injection of 32 mg/kg ACR and 4mMoles/kg HEM, the rate was equally reduced 6.1% and 8.7%, respectively. The rate of 50 mg/kg ACR and 4mMoles/kg HEM produced a biphasic decrease in capacity of transport. At 1.5 and 20 hours, a 36, 32 and 33% reduction was observed, but at 8 hours and 24 hours only 9.4 and 2.7% reductions were observed. Two to ten operinded rats appeared normal. The integrity of the BBB was evaluated using an intracerebral injection of horseradish peroxidase (HRP). 30 seconds after injection, the rats were decapitated, and the brains removed and examined for HRP leakage. Within the cerebral cortex the BBB appeared to be intact in all animals, however in animals with both bile duct ligation and multiple, large leakage spots were found in the cerebellum, primarily in white matter. The BBB damage in the cerebellum suggests that liver disease and dietary proteins may play a role in the cerebellar damage seen in alcoholic patients.

492.8

The in vivo effects of lindane (5, 10 and 20 mg/kg), RO5-4864 (5, 10 and 20 mg/kg) and picROTOXIN (PTX: 0.5, 1 and 2 mg/kg) administered i.p. in DMSO (0.5 ml/kg) in the rat were compared because of in vitro evidence that both bind the picROTOxinin site of the GABA-activated chloride channel. All 3 drugs produced similar types of seizures activity, hypothermia with peak effect at 1 hr, and hypophagia. Hypophagia potent of the three in producing seizures and hypothermia and the least effective in reducing food intake. The potencies of the 3 drugs were compared in both males and females. Thus, due to the 3 drugs shared a similar profile of effects in vivo, some differences in the time course and relative effectiveness on the various measurements could be demonstrated.
NEUROTOXICITY V  FRIDAY AM

492.9 DIFFERENTIAL SUSCEPTIBILITY OF SHORT- AND LONG-SLEEP MICE TO BRAIN NEOUROTOXICITY FOLLOWING INTRASTRIAL ALCOHOL EXPOSURE. C.R. Goodlett, M.D. Gilliam, J.M. Nichols and J.R. West, University of Iowa, Iowa City, IA 52242.

The long-sleep (LS) and short-sleep (SS) lines of mice are selectively bred for high and low sensitivity as adults to the hypnotic effects of alcohol. The two lines may also differ during development to the neurotoxic effects of prenatal alcohol exposure. Evidence from behavioral studies following prenatal alcohol exposure suggests that LS mice are more susceptible to developmental behavioral deficits than SS mice. The present study examined brain size in adult mice of the two lines given gestational treatment with alcohol. LS and SS dams were treated with gestational alcohol (either 3.0, 4.0, or 4.5 g/kg twice a day), with an isocaloric control solution (sucrose or malose-dextrin), or served as un.injected controls. For the LS offspring, there was significant, dose-dependent reduction of brain and body size in adults prenatally exposed to alcohol. For the SS offspring, prenatal alcohol exposure had no significant effects on adult brain and body size. Interestingly, the use of sugar solutions as controls resulted in significant reductions in brain size in the SS (but not LS) lines, compared to uninjectated controls. The severity of fetal alcohol effects on the developing CNS may depend in part on genetically determined differences influencing sensitivity to alcohol of the adult CNS. (Supported by NIAAA grant AA07313 to J.R.W. and AA06939 to DSM)


Following oral administration, methyl iodide (MeI, human neurotoxin) is metabolized to Methylglyoxal bis(thiosemicarbazone) and further hydrolyzed to S-methyl-cysteine, glutamate (Glu) and glycine (Johnsen & Jorgensen, 1970). The purpose of the present work was to determine whether MeI neurotoxicity could be induced in vitro by the parent compound or selected metabolites, and whether these induced neurotoxicity. Nature (15-21 days in vitro) dissociated murine (CD-1 strain) neocortical cultures were exposed to a range of MeI concentrations for 5 min in I day: cytotoxicity was assayed 24-36 hr later. Cell death was dependent upon exposure concentration and duration, with a neuronal ED50 of 500 µM and <10% MeI for 5 min and 24 hr exposures, respectively. Although MeI was the only metabolite product to induce neuronal injury, MeI was not believed as being mediated without the neuropeptide (or not induced by) Neuropeptide (1, 750, 1987), but the glutamate antagonist DL-2-amino-3-phosphonovanoic acid did not protect against the MeI induced injury and (c) cytotoxicity to MeI but not to MeI was reduced by simultaneous addition of reduced glutathione. These results indicate that MeI neurotoxicity probably results from interaction with the parent compound and (or unknown metabolites) with protein sulphydryl groups in brain tissue.


Type I and Type II pyrethroids both increase sodium channel conductance (e.g., permethrin, CPM) leading to enhanced nerve excitability and repetitive firing. Type IIs (e.g., deltamethrin, DLT) producing nerve excitability by depolarisation block. Type IIs pyrethroids have also been reported to decrease inhibition by antagonism of GABA function. The functional impact of pyrethroids on the CNS may depend on the relative contribution of these contrasting actions. Electrical kindling of the amygdala in rats was assessed in an attempt to dissociate the net effect of these mixed actions. CPM (15 mg/kg, po) or DLT (6 and 10 mg/kg, po) were administered in 1-hour periods prior to daily kindling stimulation (60 Hz, 1-s train biphasic squarewaves at 200 µA) and the rate of development of generalized seizures was compared with controls receiving the control vehicle. DLT (10 mg/kg) facilitated the development of kindled seizures. Additionally, in the absence of stimulation, 3 of 14 animals treated with DLT showed spontaneous seizures following the 3rd-5th administration of DLT. Subjects treated with CPM did not differ from controls. These data stand in contrast to a previous report in which both Type I and II pyrethroids decreased CD-50 for PTZ (Dewaud et al., 1986). DLT may enhance amygdalar kindling through an antagonism of GABA-mediated inhibition.


Superoxide dismutase (SOD) levels in CNS are critical in protecting neural function from free radical toxicity after CNS injury (mechanical, ischemic, neurotoxic or metabolic). SOD is often lessened in which involve enzymatic or non-enzymatic generation of superoxide radicals (O2\textsuperscript{-}) and their interaction with the suitable detector. We report a potent enzymatic generation of oxygenate-state levels of O2 is achieved by the interaction of phenazine methosulfate (PMS) with NADH generated by the alcohol dehydrogenase system. The O2 generation was monitored (540nm) as nitroblue formazan formation. The reaction was linear over 5min and showed a stoichiometric relationship with NADH production and NBT reduction. Under these conditions saturation level of O2 was maintained with 1-6U of SOD (bovine erythrocyte) and used for calculation. This rapid & reproducible method determines 0.1U of SOD (2mg wet wt of tissue) using a linear rate of reaction. SOD was determined in extracts (0.1M PO4 buffer, pH 7.8) obtained from different anatomical regions of rat brain. The levels correlated with the regional catecholamine turnover consistent with the possible free radical formation during neuronal activity.

492.13 TIME COURSE OF QUINOLINIC ACID-INDUCED NADPH-DIAPHORASE-CONTAINING NEURON CELL DEATH IN RAT STRIATUM. C.M. Wray* and R.J. Boegman (SPON: C. Romero-Sierra). Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Previous studies have shown that intrastriatal injections of excitotoxins destroy NADPH-diaphorase-containing neurons. We sought to establish the time course and dose-response relationship of neuronal cell death following intrastriatal injections of quinolinic acid (QUIN). Diaphorase-positive neurons appeared swollen and sometimes showed marked enlargement of the soma. We also observed in a full series of rats, which had developed with no diaphorase-positive neurons in the core injection area. A dose-response relationship was established by correlative analyses of QUIN into the striatum and determining the number of diaphorase-positive neurons. Injections of 2.5 nmol resulted in a diaphorase cell loss of 78.6%, while 7.5 nmol only 13% remained diaphorase-positive. Our results clearly indicate that in the core injection area which most closely represented the area of the injected dose of excitotoxin, NADPH-diaphorase-containing neurons are very sensitive to the neurotoxic action of QUIN.

492.14 QUINOLINIC ACID STRIATAL LESIONS DIFFERENTIALLY AFFECT MALE AND FEMALE RATS. E.M. Zubrzycki, A.B. Norman, M.L. Sherlin, and P.R. Sanberg. Laboratory of Behavioral Neurosciences, Dept. of Psychiatry, Psychology, Anatomy, and Neurology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Excitotoxic lesions of the striatum in male rats result in temporary marked weight loss resembling Huntington's disease (H-D) (1). Conversely, it was reported that striatal kainic acid lesions in female rats led to increases in weight and consumatory behavior and further, that decreases in dopamine density were predictive of the resulting weight gain (2). The present study utilized the excitoxic, quinolinic acid, lesion model in male and female rats and six hours after CNS injury (mechanical, ischemic, neurotoxic or metabolic). Although females recovered more rapidly, they did not demonstrate increased weight gain as compared to controls. Ovariectomized rats showed a pattern of weight loss that was not a compromise between male and female, and did not differ from either group. Weight gain observed in control groups did not significantly differ. These results confirm a differential response to excitotoxin lesions between sexes.

Possibility for Neurotoxicity of Fluoroacetate and Fluorocitrate

C. S. Horneff, A. A. Larson, Department of Veterinary Biology, University of Minnesota, St. Paul, MN, 55108.

The mechanism by which fluoroacetate (FA) and fluorocitrate (FC) exert their neurotoxic effects is unknown. It is known that FA is metabolized to FC, a Krebs Cycle inhibitor which causes the accumulation of citrate. Chelation of calcium by accumulated citrate in FA-poisoned animals has been proposed to be responsible for some of the peripheral effects of FA toxicity. To examine the effects of FA and FC in the CNS, and determine whether chelation of calcium is responsible for the convulsant effects of FA, we injected FC intracereally (IT) in mice. Seizures occurred almost immediately after either FA or FC at the upper end of the dose-response curve. Intracerebroventricular injection of FC also produced seizures but required higher doses and occurred only after a latent period of greater than 40 min. This confirms previous work in the cat and rat suggesting that the spinal cord is 300-400 times more sensitive to FC than FA, we found FC to be 2600 times more toxic than FA when injected IT in mice. To determine whether chelation of calcium is capable of producing similar actions to those observed after FA and FC, we injected mice IT with citrate, EDTA and EDTA. Each agent produced behavioral effects similar to those seen after FA and FC, both in onset and in motor potency. Coadministration of IT calcium or magnesium with FC or EDTA delayed the onset of seizures after FC and increased the dose of EDTA required to elicit seizures. These results suggest that these agents exert their CNS toxicity by decreasing the concentration of ionized calcium in the spinal area. (Supported by Grants DA404909, DA00124 and DA04190)

Early Activity Decrements in Grouped and Isolated Mice After Exposure to Ionizing Radiation


The effect of gamma photon irradiation on locomotor activity was examined in male Swiss-Webster mice that had been pre-housed individually or in groups of 10 for 4 wk prior to radiation exposure. Mice in each pre-housing condition received 10 Gy cobalt-60 radiation (1 Gy/min) or a sham irradiation procedure, forming 4 groups (isolated-sham, grouped-sham, isolated-irradiated, and grouped-irradiated) (N=12/group). Locomotor activity (ambulation, rearing) was monitored individually for 12 hr postirradiation (PR). Radiant activity decrements from 65 min to 4 hr PR in both grouped and isolated animals. At 4-6 hr PR, activity in the irradiated groups was only 25% of that in sham irradiated animals (the largest decrement observed). The percent decrease in the activity of irradiated animals (compared to control groups) was similar in both grouped and isolated mice, however, the absolute amount of the activity decrement produced by radiation was smaller in grouped mice due to the lower activity of the grouped control subjects (40-60% of isolated controls 2-7 hr PR).

Alzheimer’s Disease: Transmitters

Cerebral Cortical Cholinergic Reduction in Dominantly-Inherited Dystonic-Cerebellar Atrophy (OPCA) Implications for the Cholinergic Hypothesis of Dementia


Much of the experimental evidence suggests that a brain cholinergic reduction may underlie the dementia of Alzheimer’s disease (AD). We measured the behaviour of the cholinergic marker enzyme choline acetyltransferase (ChAT) throughout the brain of four patients from one OPCA family. ChAT activities were markedly reduced (-50 to -80%) in all (n=27) examined cerebral cortical subdivisions. However, in contrast with observations in AD brain, ChAT levels were generally normal and some mild increases were noted. Neuronal immunocytochemical analysis revealed a severe loss (-70%) of cholinergic cell bodies throughout the nucleus basalis but with a relative sparing of the dorsal and tangerine. Since our OPCA patients had only mild cognitive impairment we conclude that a severe cortical cholinergic loss alone is not sufficient to account for the cognitive and deterioration of advanced AD. OPCA may represent a non-AD patient group having a distinctly different pattern of disease. This may be useful for studies of behavioural consequences of a more selective cholinergic lesion. (Sup. by MRC of Canada).

Reduction in Somatostatin and Choline Acetyltransferase in Rat Brain Following Quinolinic Acid Administration Into the Lateral Ventricle

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Recent evidence suggests that certain neurodegenerative diseases like Alzheimer’s disease can be mediated by the neurotoxic action of excitatory amino acids (EAAs). In the present study, we have examined the effect of QA administration in rats into the lateral ventricle. We examined two injections of 10 ug QA for 7 days and a continuous infusion of 80 ug in 14 days were the two administration techniques compared. The percent change in SRIF content of QA for 7 days did not differ between QA in vivo or QA in vivo. QA administration into the lateral ventricle resulted in a significant reduction in CAT activity in brain. The QA infusion, however, resulted in a significant reduction in CAT activity in the R anterior cortex (0.391 ± 0.060 pg/mg prot vs 0.636 ± 0.070, p<0.05) and the R striatum (0.955 ± 0.072 vs 1.238 ± 0.074, p<0.05), compared to the controls. A reduction in SRIF content was obtained in the R anterior cortex (0.391 ± 0.060 pg/mg prot vs 0.636 ± 0.070, p<0.05) and the R striatum (0.955 ± 0.072 vs 1.238 ± 0.074, p<0.05). We also observed a reduction in the SRIF content in the R posterior cortex (0.391 ± 0.060 pg/mg prot vs 0.636 ± 0.070, p<0.05). SRIF content in the tissues from the left side (non-cannulated) were not significantly affected by the administration of QA. These data suggest that a heuristically effective model of AD could be developed by the technique of continuous QA infusion into the ventricular system.
ALZHEIMER'S DISEASE: TRANSMITTERS

ALZHEIMER'S DISEASE: SELECTIVE LOSS OF M1 RECEPTORS IN HIPPOCAMPUS AND INCREASES IN M1 AND M2 RECEPTORS IN STRIATUM AND NUCLEUS BASALIS

N. Leskow1, S. Rosenweig2, A. Winokur1 and J. N. Joyce, Departments of Psychiatry and Pharmacology, University Pennsylvania School of Medicine, Philadelphia, PA.

We have employed quantitative autoradiography to examine the pre- and postsynaptic components of the muscarinic cholinergic system in several regions of postmortem brain tissue from 4 Alzheimer's cases and age-matched controls. The density of M1 receptors ([(3H)Nephrine-HCl] increased in hippocampus and ncl. basalis of AD, whereas the density of M2 receptors ([(3H)methylscopolamine with excess pirenzepine) was not changed. No changes in the density of either subtype were observed in striatum. Within both M1 (ipsilateral) and M2 (contralateral) regions were significantly increased in density as compared to controls. Patches of very dense binding of M1 and M2 receptors were observed within the nucleus basalis of Metyer in the Alzheimer's cases, but not in the controls. This density of cholinergic loss in the Alzheimer's disease is not restricted to the cholinergic system of the hippocampus.

Analysis of dopamineergic and serotonergic systems is currently being pursued.

Funded by NIH NS01957 to AW and a grant from the American Federation of Aging Research to JN.

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Analysis of dopamineergic and serotonergic systems is currently being pursued.

Funded by NIH NS01957 to AW and a grant from the American Federation of Aging Research to JN.

Increasing evidence suggests that much of the subcortical pathology of Alzheimer's disease (AD) may be due to trans-synaptic retrograde degeneration of neurons projecting to affected cortical areas. The same may be true of neurons projecting to affected subcortical nuclei. We measured neuron density in the locus ceruleus (LC) of autopsied brains of neurologically normal individuals and AD patients. Neuron density in the LC of AD cases was significantly reduced to approximately 50%. We also observed choline acetyltransferase (ChAT) activity as a marker of cholinergic LC afferents. ChAT activity was reduced by about 50% in AD cases. Significantly, the loss of LC neurons was highly correlated with loss of pre- and postsynaptic ChAT activity. We measured the effect of LC extracts on mitogen activity in 3T3 cells as a nonspecific measure of trophic factors. Mitogen activity was significantly reduced (50%) in the AD group. Mitogen activity was significantly correlated with ChAT activity and the density of neurons in the LC in all cases. These data are consistent with the hypothesis that trans-synaptic retrograde degeneration of cholinergic projections to the LC occurs in AD.


In a sample of six Alzheimer Disease (AD) cases and six age-matched controls, we have determined the extent of neuronal loss in the locus ceruleus (LC) and compared differences between diseased and control tissues with regard to pathology and changes in β-adrenergic binding in the frontal and temporal lobes. In the AD cases, neuronal density in the LC was reduced by 30-50%. In the frontal cortex, a 20-30% decrease in neuronal density was observed. Using drugs that selectively mask either the β1- or β2-receptor subtypes, significant increases in the density of both β1- and β2-adrenergic receptors were seen. The increase in β1-receptor binding was localized to the deeper cortical lamina, while increased β2-receptor binding was more generally observed throughout the cortex. In a larger sample, these findings were confirmed by homogenate binding. Since these increases in β-adrenergic binding were seen in both homogenate and autoradiographic experiments, and changes in receptor subtype were regionally selective, these changes indicate that the cortex can still exhibit a plastic response to noradrenergic denervation in AD.

493.12 LOBULUS COERULEUS CELL LOSS IN ALZHEIMER'S AND PARKINSONIAN PATTERN ANALYSIS. K. F. Park, D. S. Tabaton, P. R. Kalaria and J.R. Unnerstall. Departments of Neurology and Pathology, Case Western Reserve University Sch./Med., Cleveland, OH 44106.

In a sample of six Alzheimer Disease (AD) cases and six age-matched controls, we have determined the extent of neuronal loss in the locus ceruleus (LC) and compared differences between diseased and control tissues with regard to pathology and changes in β-adrenergic binding in the frontal and temporal lobes. In the AD cases, neuronal density in the LC was reduced by 30-50%. In the frontal cortex, a 20-30% decrease in neuronal density was observed. Using drugs that selectively mask either the β1- or β2-receptor subtypes, significant increases in the density of both β1- and β2-adrenergic receptors were seen. The increase in β1-receptor binding was localized to the deeper cortical lamina, while increased β2-receptor binding was more generally observed throughout the cortex. In a larger sample, these findings were confirmed by homogenate binding. Since these increases in β-adrenergic binding were seen in both homogenate and autoradiographic experiments, and changes in receptor subtype were regionally selective, these changes indicate that the cortex can still exhibit a plastic response to noradrenergic denervation in AD.


We measured the concentration of the neurotransmitter noradrenaline (NE) in autopsied brain of ten infant children (aged 3 months to 12 years) and four adults (aged 22 to 59 years) with Down's Syndrome (DS). This study was prompted by evidence demonstrating 1) markedly increased risk for the early development of AD in the brain neuropathology; 2) clinical changes of Alzheimer's disease (AD) and 2) reduced levels of NE in AD cerebral cortex and hypothalamus. Brain histological analyses revealed the typical changes in AD (atrophy, neurofibrillary tangles and neurites) in the three oldest DS individuals (ages 43, 55, and 59 years). When compared with age-matched controls, NE levels were markedly reduced. This was true of all three oldest DS cases. The magnitude of this reduction was similar to that observed in AD brain. Mean NE levels in young DS cases were comparable to levels in the normal brain (5 - 80 ng/g).


The amygdala often has the highest density of Senile plaques (SP) in Alzheimer's disease (AD) brain but the relationship of SP distribution to defined neurons in the amygdala is not well understood. We used immunohistochemical procedures to examine somatostatin (SS), neuropeptide Y (NPY), cholecystokinin (CCK), and substance P neurons in the amygdala of 6 AD patients and 6 age-matched controls. SP were defined with Thioflavine S and the silver method of S.B. Campbell et al. (Soc Neurosci Abst 13:678). SP are found in all nuclei of the amygdala without a clear mediodorsal gradient. The ventral region and paralaminar nucleus are relatively spared. SS and NPY neurons are present in the dorsal portion of lateral (L), accessory lateral (AL), and lateral central (LC) nuclei and the periamygdaloid cortex (PAC). Morphologically these neurons are aspinystellate cells (Class II). In AD penkalpykral density is not associated with SP distribution. Reduced and neurites in SP are seen with much greater frequency in the cortical and superficial AB nucleus. CCK neurons are found in substantial numbers in the AB and PAC where they contribute to SP formation. Substance P neurons are present in AB, CL PAC and dorsal basal magnocellular nucleus where they significantly contribute to SP. Despite the occurrence of SP throughout the amygdala only SP in medial regions contain peptideric fibers. This differential incorporation of defined fibers into SP may reflect differences in the structure of SP in different regions of the amygdala.
THA INCREASES ACTION POTENTIAL DURATION OF CENTRAL HISTAMINE NEURONS IN VITRO. P.B. Reiner and E.G. McGee, Kinsey Laboratory, Dept. Psychiatry, Univ. of British Columbia, Vancouver, BC Canada V6T 1M5.

THA (9-amino-1,2,3,4-tetrahydrossoaacidine), has been reported to produce marked clinical improvement in patients suffering from Alzheimer’s disease (AD). THA has anticholinesterase activity and so might alleviate the well-documented deficit in cortical cholinergic innervation in AD. However, THA appears to be clinically more effective than other anticholinesterase agents, and thus other mechanisms might play a role in its therapeutic efficacy.

Intracellular recordings were obtained from histaminergic intralaminar thalamic neurons, and THA (10 µM) was found to bind with an affinity of 27 ± 8 nM (mean ± SEM); capacity (Bmax) of 1.09 ± 0.10 pmol/mg protein and was reversible in the presence of THA. THA is the first demonstration of specific, saturable, and reversible binding of [3H]THA to membranes prepared from human brain. These [3H]THA binding sites are likely to be physiologically significant receptors.

Data on brain regional distribution in subjects with Alzheimer’s disease and age matched controls will also be presented. Supported by the MRC, OMHF and OMH.


The memory dysfunction of Alzheimer’s disease has been associated with a cortical cholinergic dysfunction. In the rat, the ibotenic acid lesion of the nucleus basalis magnocellularis (NBm) reduces choline-acetyltransferase activity in the dorsolateral frontal cortex (DFC), medial prefrontal cortex (MPFC) and parieto-temporal cortex. The present study examined influence of ibotenic acid lesion of DFC, MPFC on retention of conditioned avoidance learning (DAL). Male Wistar strain rats (8 weeks old) were housed in a air-conditioned room (23±1°C) with 12:12 light cycle (light on at 0700) under a 6% humidity. In the conditioning, two pure tones with 800 Hz for positive conditioned stimulus (CS) and 400 Hz for negative CS were used as irrelevant stimuli. The retention of DAL was examined on 7, 10, 14 and 21 days after the surgery. The DFC lesion impaired the retention accompanied by increased NR and decreased PR similar to an excess of unlabelled IP3. This local dysfunction of cortex region related with NBm elicits impairment of learning behavior.

THA MODULATION OF PHOSPHOMONOSTER METABOLISM IN THE HIPPOCAMPUS OF Alzheimer’s DISEASE. C. Barbagallo, J.E. Bradstreet, D. McCracken and J.W. Petreger, Jr., Deps. of Behavioral Neuroscience & Psychiatry & WPC, University of Pittsburgh, Pittsburgh, PA 15260.

The phosphomonoester phosphoethanolamine (PEA), a key intermediate in the biosynthesis of membrane phospholipids, is released spontaneously and in a stimulus (high K+ or kainate) dependent manner from hippocampal neurons. In addition, 31P NMR spectra demonstrated elevated levels of PEA in Alzheimer’s diseased brains. To investigate the effects of THA on hippocampal metabolism, extracellular recordings were made from rat hippocampal slices (3 month old Fischer 344). The amplitude of population EPSPs evoked during the Schaffer collateral/commisural input to CA1 neurons was monitored prior to and during a 30 min bath application of varying concentrations of PEA. At 1 mM and 2 mM PEA induced a substantial depression of population EPSP amplitudes (43% +/- 21% depression, n=8). This depression was only partially reversible following extended time periods (87% +/- 21% of baseline at 30 min, n=8). 31P NMR spectra from freeze-clamped hippocampal slices revealed no effect of PEA on high-energy phosphate or membrane phospholipid metabolism. We are currently studying this regulation with intracerebral recordings. Supported by ROI-AG05637, AG-03133-DIA, and ROI-MH4158 to J.W.P., and an RCDA (NS01196) to G.B.
REGIONAL GLUCOSE METABOLISM ALTERATIONS WITH INTRAVENTRICULAR QUINOLINIC ACID ADMINISTRATION

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Experimental Therapeutics Branch, NINCDS, and MPFC, University of Maryland, Balt., MD, 21228

Neurodegenerative disorders may involve neurotoxic EAA transmitters like quinolinic acid (QA). In an attempt to develop an animal model of Alzheimer's disease, we have established two different administration techniques, chronic intraventricular injection (7 days) and intraventricular infusion (14 days) in the rat, to test brain metabolic changes and its similarity to AD metabolism. QA was introduced into the right lateral ventricle using either daily injection of 10 ug QA via chronic intracerebroventricular cannula for 7 days, or continuous infusion of QA using an osmotic minipump (Alzet 2002) for 14 days with a total delivery of 400 ug QA/rat. Local cerebral glucose utilization (LCGU) was measured in 55 different brain areas using the quantitative (14C)-2-deoxyglucose (2DG) autoradiographic method (Sokoloff et al., 1977), as umol glucose/100 gm brain tissue/min. Statistical analysis was done with multivariate analysis of variance. No remarkable alterations in LCGU were noted in the daily QA injection animals. Animals with the 14 day QA infusion demonstrated LCGU in a number of cortical brain areas and increases in LCGU in selected sensory processing areas.

We will compare alterations in glucose metabolism in this rat model with metabolic alterations seen in Alzheimer patients.

LEARNING AND MEMORY: ANATOMY III

LEARNING AND MEMORY DEFICITS AFTER LESIONS OF NUCLEUS BASALIS IN TURTLES, RHINEHART, J.A., Blaus*, & M. Pettilage*. Dept. of Psychology, St. John's University, Jamaica, NY 11439.

The basal forebrain of turtles contains a nucleus basalis, a group of cholinergeric cells that project to the dorsal cortex. The dorsal cortex is a three-layered cortex on the surface of the telencephalon. We compared the effects of lesions of nucleus basalis and dorsal cortex on patterns of lesion acquisition and reversal and on retention of maze learning. Lesions of the dorsal cortex were made by suction current, while lesions in nucleus basalis were made by injections of ibotenic acid. Compared to sham-lesioned controls, both lesions produced an impairment on acquisition and reversal of a horizontal-vertical discrimination. There was no significant difference between the two lesioned groups on either acquisition or reversal. Maze retention was also impaired by both lesions, but there was a suggestion that the impairment was greater in the nucleus basalis group. These results suggest that the nucleus basalis of mammals is a phylogenetically ancient structure that participated in learning and memory in the reptilian ancestors of mammals.

DIFFERENTIAL ANATOMICAL PROJECTIONS BETWEEN FRONTAL CORTICES AND THE BASAL FOREBRAIN IN THE RAT. D.R. Beers*.

The importance of a neural region in a particular psychological function (e.g. memory) can be inferred after psychometric testing is directly related to changes in glucose utilization in experimental testing is completed. We used magnetic resonance imaging (MRI) to determine the limits of experimental lesions in the rhesus monkey brain and in unlabeled rats using the brain in vivo due to its soft tissue contrast and the ability to obtain images in three dimensions. Monkeys were appropriately anesthetized and labeled with a magnetic stereotactic instrument, and imaged using transverse T-1 weighted images, sagittal T-2 weighted images, and coronal T-2 weighted images. In contrast, injections in the horizontal nucleus of the diagonal band labeled neurons in the medial prefrontal cortex. Conversely, when bis-Benzimide was injected in the media prefrontal or dorsolateral frontal cortices, labeled neurons were seen in the horizontal nucleus of the diagonal band or the nucleus basalis magnocellularis, respectively. Identification of these basal forebrain areas was confirmed by the AChE stain. These results indicate that the basal forebrain of the rat receives reciprocal differential anatomical projections and this suggests that these connections may mediate differential behavioral functions.
LEARNING AND MEMORY: ANATOMY III

5-40 minutes following presentation of ten 0.6 mA footshocks (shock sensitization). This facilitation represents an unconditioned effect of shock on startle. These data suggest that even though NBM lesions produce a taste aversion learning deficit that parallels impairments seen in animals with basolateral amygdala lesions, this deficit does not appear to be mediated by cholinergic projections to the amygdala. It is suggested that NBM lesions might have damaged other cholinergic systems or that some other neural transmitter projecting to amygdala might be involved.

DIFFERENTIAL EFFECTS OF QUISQUALATE OR IBOTENATE INJECTED INTO NUCLEUS BASALIS MAGNOCELLULARIS (NBM) ON BEHAVIOR AND NEUROCHEMISTRY. G. Henrik, A. Markowska* and D.S. Ott.. The Johns Hopkins University, Baltimore, MD 21218.

Lesions of the NBM produced by either quisqualate (QUIS) or ibotenate (IBO) should produce different effects on behavior and neurochemistry in rats. IBO injections decreased choline acetyltransferase (ChAT) activity (48%) and [3H]Neurotensin binding (45%) in frontal cortex and impaired performance in T-maze alternation. QUIS injections decreased CHAT activity (78%) but did not alter [3H]Neurotensin binding or impair performance. Therefore, non-cholinergic mechanisms that kill the NBM project to cortex, and have presynaptic [3H]Neurotensin binding sites may be involved in the performance deficits. These data have implications for the effects of loss of NBM cells and provide new information about the neural organization of cells in the NBM and the mechanisms responsible for their degeneration in Alzheimer's Disease.


The amplitude of the acoustic startle reflex can be markedly increased during the 5-40 minutes after presentation of ten 0.6 mA footshocks (shock sensitization). This facilitation represents an unconditioned effect of shock on startle. A consistent literature indicates that the amygdala plays an important role in unconditioned as well as conditioned responding to fear-predicting stimuli. Lesions of the central nucleus of the amygdala, but not of the immediately adjacent lateral nucleus, blocked shock sensitization. Central nucleus lesions also decreased reactivity to shock (jumping and flinching). However, this did not account for the blockade of shock sensitization, because when a higher shock intensity was used, central nucleus lesions were effective at both lower intensities. Central nucleus lesions still blocked shock sensitization.

A follow-up study was conducted to determine the central nucleus efferent pathway that mediates shock sensitization of the startle. Lesions of the rostral part of the ventral amygdalofugal pathway (VAF), which carries efferents to the forebrain, had no effect on shock sensitization of startle. In contrast, lesions of the caudal part of the VAF, a branch of which projects directly to the startle circuit, blocked shock sensitization of startle.

These findings, along with previous results, lead us to hypothesize that activation of the central nucleus of the amygdala increases startle through its projection to the startle pathway, and this mediates the unconditioned effects of shock on startle, as well as the conditioned effects of stimulus paired with shock on startle.


Lesions of the nucleus basalis magnocellularis (NBM) deplete cortical acetylcholine levels and produce memory impairments in rats. While GM, a cholinergic agonist stimulates the recovery of those levels after lesions of the NBM (Florian et al. Neurosci. Lett. 75:311-316, 1987). In the present study, electrolytic and ibotenate lesions of the NBM produced deficits in the Morris Water Maze. GM-treated rats were not significantly impaired by electrolytic lesions on this task. Both electrolytic and ibotenate lesions impaired acquisition of a passive avoidance task; however, GM treatment facilitated performance of ibotenate-lesioned rats only. Locomotor activity, measured in an open field, was not affected by NBM lesions or by GM administration. Although the two lesion types did not differ in terms of the number of NBM cells destroyed, the electrolytic lesions produced greater damage to adjacent structures. GM treatment did not affect cell survival in the NBM after electrolytic or ibotenate lesions.

Further correlations between behavioral and neuroanatomical data will be reported.

Supported by grants from Fidia Research laboratories and S. & C. Del Duca Foundation.

NUCLEAR BASALIS LESIONS AND PAVLOVIAN CONDITIONING IN THE RABBIT. S.H. Gim and D.A. Powell. Born VA Hospital and University of South Carolina, Columbia, SC 29201.

Ibotenic acid (IA) lesions of the NBM were made prior to classical conditioning in rabbits; other rabbits were subjected to sham lesions of the NBM, served as unoperated controls, or received pseudoneconditioning (viz., random shocks/tones). Electrolytic and heart rate (HR) conditioned responses (CRs) were assessed. Lesions of the NBM had no effect on acquisition of the EB CR, but the HR CR of the lesioned animals was attenuated compared to the control groups. IA lesions of the NBM reduced cortical CAT concentrations by 50% compared to control animals. In a second experiment, rabbits received sham or NBM lesions after conditioning training was completed, but prior to retention testing. There were no differences between the groups for the EB or HR CRs during either phase of this experiment, even though IA lesions reduced cortical CAT concentrations by 80%.

These results suggest that the NBM may be part of a cortical-subcortical pathway that modulates Pavlovian autonomic conditioning. The effects of the lesions of the NBM were most apparent early in conditioning, suggesting that the NBM affects attention/stimulus registration processes during learning.
The amygdala is involved in the expression of conditional analgesia. F.J. Helmstetter, R.N. Leaton, M.S. Fanselow, & D.J. Calabresi. Psychology Department, Dartmouth College, Hanover, N.H. 03755.

The amygdala seems to play an important role in the acquisition and performance of conditional fear as reflected in a number of preparations. We wished to determine if this structure is involved in the expression of fear displayed by rats in the presence of shock associated stimuli. Relative to controls, animals with electrolytic lesions involving portions of the central, lateral, and basolateral amygdaloid nuclei were less time freezing and were less anergic as indexed by the formalin test. Bilateral microinjection of diazepam (30µg /1µl x2) into the amygdala produced a similar pattern of results. These data are consistent with the position that conditional analgesia is a response to a central fear-like motivational process.


Acoustic stimuli are transformed into symbols of danger by way of projections from the acoustic thalamus to the amygdala. The relay to the amygdala should, therefore, originate in thalamic areas that are within the projection field of the inferior colliculus (IC). In the present study we determined whether the central (CA) and/or lateral (LA) amygdaloid nuclei receive inputs from neurons that receive afferents from the IC. WGA-HRP was delivered iontophoretically to CA (n=5) or LA (n=10). Injections confined to LA resulted in transport to cells in the posterior intralaminar nucleus (PIN) and medial division of the medial geniculate body (MGM). Following injections in CA labelled neurons were located in the posterior thalamic region (PT) between the MGM/PIN and the anterior pretectal nucleus. Injection of WGA-HRP into the MGM/PIN (n=2) resulted in retrograde transport to cells in the shell regions of IC and anterograde transport to LA. Injections in PT (n=4) failed to label cells in IC, but did produce anterograde transport to the CA. Injections of IC (n=5) produced anterograde transport to the MGM/PIN, but not to PT. These observations demonstrate that neurons in the MGM/PIN receive inputs from the IC and project to LA. In contrast, CA does not appear to receive a direct input from the acoustic thalamus. The projection to LA may, therefore, be a critical relay in emotional learning. (Supported by MH38774).


The amygdaloid central nucleus (ACE) is known to play an important role in classically conditioned heart rate responses to acoustic stimuli in the rabbit (Kapp et al., 1979; Gentile et al., 1986). While the efferent projections of this nucleus to various cardiovascular structures have been examined extensively in this and other species, afferent projections to the ACE have been described in only one study in the rabbit (Kapp et al., 1984). The present study examined efferent afferent projections to the ACE and other amygdaloid nuclei, with a focus upon inputs from auditory nuclei.

New Zealand rabbits received injections of the retrograde tracer Fluoro-gold (4% in saline, 20nl) into the ACE. Two to three weeks later, the animals were perfused and sections were examined, using fluorescent microscopy, for the presence of retrogradely labelled cells in subcortical structures. Labelled neurons were located primarily ipsilaterally, in several areas including substantia innominata, midline thalamic nucleus, ventromedial and paraventricular hypothalamus, dorsal and ventral periaqueductal gray region, medial parabrachial nucleus, substantia nigra, locus coeruleus, and nucleus tractus solitarius. Of particular interest were labelled neurons in the magnocellular region of the medial geniculate nucleus and in the ventrolateral parabrachial region bordering on the intermediate nucleus of the lateral geniculate. Both groups of cells may relay auditory or multi-modal sensory information to the ACE. Supported by NIH grants NS 24874, HL 07426, and HL 36588.


Anatomical tracing studies indicate that the medial geniculate body (MGB) projects to areas of the amygdala and caudate-putamen, as well as to the auditory cortex. Responses of single neurons to electrical stimulation of the MGB were examined in those subcortical areas that receive direct anatomical projections from the MGB. Recordings were made in rats (n=20) anesthetized with chloral hydrate (7.5%, iv). Units in the caudate-putamen and lateral amygdala were excited by short pulses (35 µsec each, 200 µsec apart, 500 µA, 0.1 Hz). Mean latencies were 3±2.1 msec in the lateral amygdala (n=61), 3±2.2 msec in the medial caudate-putamen (n=46), and 7±2.6 msec in the lateral amygdala (n=35). Units in other amygdaloid regions required longer, higher frequency pulses (single shock, 500µsec, 500 µA in the central amygdala and 1.3 mA in the basolateral/basomedial amygdala, with frequencies varying from 0.2 to 1 Hz). Mean latencies were 9±2.6 msec in the central amygdala (n=38) and 13±3.2 msec in the basal amygdala (n=50). In the latter regions, responses followed a caudo-rostral gradient, with cells responding to MGB stimulation being more numerous caudally. These data are consistent with anatomical findings demonstrating direct projections to the lateral amygdala and caudate-putamen. The latency and higher threshold responses in the central and basal amygdaloid nuclei may be accounted for by multisynaptic projections to these areas, perhaps from one of the regions receiving direct MGB projections. Supported by NIH and MH38774.


 Destruction of the medial geniculate body (MGB), but not its nocortical projection field, disrupts the classical conditioning of autonomic activity (increases in heart rate and respiratory rate) and emotional behavior ("freezing", F) to a pure tone paired with footshock. However, cortical areas along the rhinal fissure, which were spared in previous lesion studies, also receive inputs from MGB. In the present study we therefore examined whether combined removal of peripheral and neocortical projection areas would affect emotional (fear) conditioning. Rats were lesioned, allowed to recover for 3-5 weeks, subjected to fear conditioning, and tested. Conditioned responses did not differ in controls (AP, 16±2; F, 11±2; n=7) and in rats with cortical ablations (AP, 13±1; F, 9±2; n=6). In contrast, conditioned responses were significantly reduced by lesions of MGB (AP, 4±1, p<0.01; F, 0; pc<0.01; n=7). Conditioned responses were similar in animals with MGB lesions to controls in responses in controls given random presentations of tone and shock (AP, 4±1, n=5; F, 4±2, n=5). Two between-subjects measures: that fear conditioning does not depend upon any cortical area receiving inputs from MGB. Since the MGB is necessary for the formation of the association between the tone and shock, its subcortical projections, which originate in the medial MGB and posterior intralaminar nucleus, must be essential components of the fear conditioning circuitry. (Supported by MH38774).
LEARNING AND MEMORY: ANATOMY III

ANIMALS WITH ISCHEMIC OR IOTIC ACID HICPOCAPAL INJURY DISPLAY IMPAIRMENT IN WORKING MEMORY. Bryan, B.C., Winn, F., Hunsperger, R.D., et al. J. Neurosci. 11, 1985.) Injection of bicnic acid (i.e., ibotenic acid) into the hippocampus has been shown to cause severe neuronal damage. The results presented here suggest that such an injection may selectively impair working memory performance. The data also indicate that the lesioned animals may have difficulty in learning new tasks or remembering previously learned ones. These findings support the hypothesis that the hippocampus plays a critical role in the retention of spatial information.


The medullary region of the rabbit's auditory system is well known for its role in the processing of auditory information. However, few studies have been done on the afferent connections of the thalamic region of the medial geniculate nucleus (mMGN). Previous work by Ungerleider and Phillips has shown that the mGN receives input from the amygdala and the anterior thalamic nuclei. These studies suggest that the mGN is involved in the processing of affective and emotional information. The present study extends these findings to the rabbit and provides additional insight into the neural mechanisms underlying the processing of auditory information.


The radial maze is a widely used test for rodents that assesses spatial learning and memory. The present study found that pretraining trials improved working memory performance in rats. This effect was not observed in animals with bilateral hippocampal lesions. These findings suggest that the hippocampus plays a critical role in the processing of spatial information, and that learning new tasks may be impaired in animals with hippocampal damage.

THALAMIC INPUT TO MEDIAL PREFRONTAL CORTEX IN MACAQUES. K. R. L. Lin, L. A. Behnke, B. K. M. von. U. Engel, and R. W. Low. J. Neurosci. 11, 1985.) The present study examines the thalamo-prefrontal projection in Macaque monkeys. The results indicate that the projection is specific to the anterior cingulate cortex and that it may be involved in the processing of affective and emotional information. These findings support the hypothesis that the thalamus plays a critical role in the processing of affective and emotional information.
In a previous experiment, monkeys (M. fascicularis) with medial thalamic lesions were found to exhibit an acquisition deficit for a visual pattern discrimination task (A + B -). The possibility that this deficit could be due to difficulty in reversing the rule which had been practiced in an immediately preceding series of non-matching trials (win/lose, with respect to the sample stimulus) was assessed by presenting 3 additional sets of discrimination problems. Each set was composed of 8 problems learned in succession to a criterion of 90% correct in 30 trials. Practice with associative problems did not reduce the performance decrement of the experimental monkeys, and it was apparent that they were retarded in developing a learning set. A transfer task (A + C + D - B -) was also presented, in which the elements A and B had been learned as a pair and maintained the original reward assignments as members of new pairs. Monkeys with lesions were not able to maintain the learned response choice as well as normal monkeys, when the context provided novel stimuli which were differentially rewarded. These results suggest that the deficit sustained by monkeys with medial thalamic lesions is related to the acquisition and maintenance of the stimulus/reinforcement association, and is affected by the amount of within-problem interference. (Supported by VA Medical Research Funds.)

We have previously shown that electrically induced lesions of the neocortical (MM) in mice induce memory deficits in tasks based on spatial (S.A.) and on a T-maze. Subsequent experiments showed that these deficits could be alleviated by using between-trials contextual changes. This suggests that the observed impairments might stem from the automatic (as opposed to effortful) form of memory involved in S.A. The present experiments compared the effects of ischemic acid lesions of MM in either sequential (successive trials separated by intertrial intervals ranging from 30 sec to 3 min) or delayed (2 forced trials followed by a test free trial given up to 6 hours) procedures using S.A., reinforced win-shift or win-stay protocols. Results showed that when tested with the win-shift rule, MM-lesioned mice still exhibited deficits compared to controls but with longer intervals than in spontaneous alternation. The use of the win-stay rule, however, produced no observable deficit in contrast to that seen in S.A. and win-shift protocols. These results suggest that MM lesions might impair primarily automatic form of memory.

The behavior of septal lesioned and normal rats was compared on a variety of alternate route variations of the 3-table problem. Septal rats displayed impaired test trial behavior on all variations of the task. However, the route choice pattern did provide some insight into the nature of the septal deficit. Septal rats were able to distinguish and use a cue during both exploration and test trials. If available, the chosen route was usually the most direct between any two tables. When no direct route was available, the rats took the boundary route, i.e., the route which formed the peripheral edge of the apparatus. In contrast, normal rats chose the most direct route only in the simplest configurations. In more complex configurations, normal rats consistently chose the central but longer route which may have allowed delayed choice or single point reference orientation.

These results suggest that septal rats are able to store and use information about distances if not locations, and that normal rats are able to utilize reference cues and locations according to several other variables.

The purpose of this study is to investigate the general neurological deficits and learning deficits produced by the electrolytic lesions of the VM-VL. A 15 item neurological test and 3 learning tasks: T-maze spatial reversal, T-maze brightness discrimination, and complex maze, were used in this study. No deficits were observed on any of the neurological test items following the VM-VL lesions. Lesions affected performance on the T-maze spatial reversal task (Lama ratio=3.21, d F=4, p=0.0345) but not on the other two learning tasks. The results were similar to those obtained by others in studies of the GD-Pt.
494.29
STIMULATION OF THE LATERAL SEPTUM IS A MORE EFFECTIVE CS THAN THE MEDIAL SEPTUM FOR THE CLASSICAL CONDITIONING OF THE EYEBLINK RESPONSE.
B.J. Knowlton and R.F. Thompson. Department of Psychology, University of Southern California, Los Angeles, CA 90089.

Eight rabbits were trained in the classically conditioned eyeblink response procedure using stimulation of the septal nucleus as the conditioned stimulus (CS). Each rabbit was trained with both medial septal stimulation and lateral septal stimulation. Stimulation of the medial septum was a far less effective CS than stimulation of the lateral septum. The differences may be due to the different roles of these two nuclei in classically conditioning. Conditioning using lateral septal stimulation as a CS is dependent on the cerebellar interpositus nucleus, as is conditioning using peripheral and other brain stimulation CSs.

This research was supported by a National Science Foundation predoctoral fellowship to BJT and grants from the McKnight Foundation (22-1873-6988), the National Science Foundation (33-487-6578), the Office of Naval Research (N00014-83) and the Sloan Foundation to RFT.

494.31
CONCURRENT LEARNING AND RETENTION TESTED WITH REVERSIBLE COOLING OF VENTROMEDIAL TEMPORAL CORTEX. C. George, R. Cirillo, D. Chen and J. Horrel. Dept. of Psychology, Syracuse University and Dept. of Anatomy and Cell Biology, Health Science Center, Syracuse, NY 13210.

We have found a strip of cortex on the ventromedial temporal lobe (VMT) that is essential for performance of delayed match-to-sample, while the rest of the temporal cortex could replace the role. It extends from posterior parahippocampal gyrus to anterior ventral inferotemporal cortex. Previously, we had found that small inferotemporal lesions impaired learning while not recall and at the same question of VMT. VMT was covered with a single cryode on each side of monkeys that were then trained on a concurrent learning and retention task. The animals were trained on a set of four object discriminations and these were presented concurrently with four new discriminations to measure new learning and retention. The overlearned pairs were then presented together with four new discriminations while cooling VMT. The hypothesis was that with VMT suppressed, the animals would recall the overlearned discriminations but not learn the new discriminations; however, they were severely impaired in both learning and recall. This is the most severe retention deficit we have found with small temporal cortex lesions. (Supported by NINDS grant NS18291).

494.32
PARAHIPPOCAMPAL GYRUS AFFERENT CORtical CONNECTIONS AS DEMONSTRATED BY RETROGRADELY LABELLING CELLS WITH WGA-HRP. C.C. Martin-Elkins* and J. Horrel (SPON: J. Horrel). Dept. of Anatomy, SUNY Health Science Center, Syracuse, NY 13210.

Inferotemporal cortex (IT) has been shown to play an important role in acquisition and retention of visual information. While it has generally been considered a single, homogenous visual area, recent studies have indicated that the parahippocampal gyrus to anterior ventrotemporal cortex. Previously, we had found that small inferotemporal lesions impaired learning while not recall and at the same question of VMT. VMT was covered with a single cryode on each side of monkeys that were then trained on a concurrent learning and retention task. The animals were trained on a set of four object discriminations and these were presented concurrently with four new discriminations to measure new learning and retention. The overlearned pairs were then presented together with four new discriminations while cooling VMT. The hypothesis was that with VMT suppressed, the animals would recall the overlearned discriminations but not learn the new discriminations; however, they were severely impaired in both learning and recall. This is the most severe retention deficit we have found with small temporal cortex lesions. (Supported by NINDS grant NS18291).

494.33

Although combined amygdalo-hippocampal removals in macaques severely impair their performance on delayed nonmatching-to-sample (DNMS) when the delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. Such mastery cannot depend on the formation of specific visual discrimination habits, because (a) different pairs of objects is used on every trial and (b) within a trial, the reinforcement contingencies to the same object are inconsistent. To master the task in the absence of the limbic system, the animal must be able to learn a rule, which requires (i) an expansion of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of stimulus-difference-response habit. We have now found that if inferior prefrontal lesions (which produce a moderate DMMS impairment by themselves) are added to amygdalo-hippocampal lesions, monkeys lose the ability to perform DMMS even when the delays are less than 10 seconds. This finding suggests that the inferior prefrontal cortex serves one or more of the processes described above needed for rule learning, and that it does so by mediating complex set of interactions between the inferior temporal cortex and the neostriatum, with both of which the inferior prefrontal cortex is directly interconnected.

494.34
IMPAIRMENT OF SPATIAL, OLFACTORY, AND AUDITORY SERIAL REVERSAL IN AN ANIMAL MODEL OF HUMAN DEMENTIAL AMNESIA. R.L. Knoth, R.C. Hoyt, S.A. Rachmek. Department of Psychology, University of New Hampshire, Durham NH 03824.

P.J. Langgeld. San Diego VANC.

The post choline deficient (PTD) rat is an animal model of Wernicke-Korsakoff's disease, the most common cause of global demential amnesia in humans. Behavioral testing of PTD rats has revealed learning and performance deficits that are both averse and appetitively motivated spatial tasks, including spatial delayed alternation and spatial delayed non-match-to-sample, but not deficits on spatial (left/right) and visual (light/dark) discrimination (Mair et al., Brain Res., in press; Knoth et al., Neurosci. Abstr., 13, 1127). These findings suggest that specific deficits exist on spatial and visual discrimination that affect the degree to which the learning and performance deficits extended to other sensory modalities.

A total of 16 PTD and 24 controls were compared on spatial, olfactory, and auditory serial reversal (SR). On spatial SR, experimental animals required consistently more trials than controls on initial, and all subsequent reversals. On olfactory SR, experimental animals required significantly more trials than controls on initial, and all subsequent reversals. Overall, these deficits were observed in auditory SR too. These results support the global nature of the learning and performance deficits observed in PTD rats.
ALTERED EXPLORATORY ACTIVITY IN AN ANIMAL MODEL OF DENCEPHALIC AMNESIA. S.A. Babakhani; K.C. Mair; & R.L. Knott (SPON: E. Hagstrom). Department of Psychology, University of New Hampshire, Durham, NH 03824.

The post thiamine deficient (PFD) rat is an animal model of diencephalic amnesia, characterized by medial thalamic lesions and impaired performance on tasks measuring learning and memory. In this experiment, we videotaped and open field activity of 16 PTD and 16 control animals during 3 daily 15 minute sessions. On day 1 and 2, animals were placed in an empty circular field and on day 3 a novel stimulus was placed in the center. Videotapes were coded in 1 minute intervals for line crossing, a measure of locomotor activity and rearing, a behavior in which rats stand on their hindlegs and execute sniffing bouts and multiple shifts in head position.

Results showed that on days 1 and 2, PTD rats exhibited significantly more line crossings than controls. Both groups showed a decrease in the frequency of line crossing and rearing within each session. On day 3, the controls made more approaches to the novel object during the first 1 minute interval. However, controls made a rapid decrease in the frequency of these responses and PTD animals did not. This pattern of increased line crossing and decreased rearing was limited to rats with the lesion of the intralaminar thalamic nuclei typical of the PTD model.

MUSCLE: STRUCTURAL CHARACTERISTICS


The purpose of this study was to determine the fiber type composition of motor units (MU) in the cat diaphragm. MU's were classified as fast or slow (S) based on the sag test. Fatigue resistance and fatigue rate (fatigue index) was used to further subclassify fast units as FR (FI > 0.75), Fint (0.25 < FI < 0.75), or FF (FI < 0.25). MU fibers were identified using the glycogen depletion technique. Fibers were classified as type I or II based on their activity. Subclassification of IIA and IIB fibers was based on ATPase activity after acid preincubation (pH 4.2 and 4.6). Fibers belonging to units were uniformly composed of type I fibers. FR and FF fibers were composed exclusively of types IIA and IIB fibers respectively. Fint units showed a mixed fiber type composition (both IIA and IIB). Those Fint units with FI > 0.50 were comprised primarily of IIB fibers (80% vs 20% IIA, whereas Fint units with FI < 0.50 were comprised primarily of IIA fibers (90% vs 10% IIB). We conclude that, unlike the homogenous fiber type composition of S, FR, and FF MU's, Fint units are composed of a mixed population of type I fibers with proportions of IIA and IIB fibers related to unit fatigue resistance.

PREDICTION OF FIBER TYPES IN THE CAT QUADRICEPS MUSCLE. B.G. Sasso,Jr., L.L. Glenn, P.J. Behbehani. Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, Ohio 44106-3082.

Neuromuscular compartments (NMC) have been identified in various muscles of the cat hindlimb including the quadriceps. The purpose of this study was to determine if the ratio of tension at 20 Hz (P20) to tension at 100 Hz (P100) could predict the fiber type distribution in the nine quadriceps NMC.

For each NMC, single twitch tension, tension at 20 Hz and 100 Hz and fatigue index was determined. The proportion of fiber types in each NMC was correlated to the contraction properties. The average fiber type distribution (across all nine quadriceps NMCs) was SO = 35.8% (3.4 - 96.5), FOG = 22.4% (0.5 - 37.5), FO = 38.7% (2.3 - 63.6). The range of P20/P100 was 0.26 - 1.00 while the fatigue index ranged from 0.19 - 0.70. The correlation coefficients of P20/P100 were -0.94 with FOG, 0.79 with SO, -0.58 with PD.

Our findings show that P20/P100 can accurately predict the percentage of FOG fibers in NMC of the cat quadriceps.

FIBER-TYPING BY ATPase HISTOCHEMISTRY OR MYOSIN IMMUNOHISTOCHEMISTRY: EFFECT OF DENERVATION ON MUSCLES OF C57BL/6J MICE. R.L. Davis and G. Desypris. Dept of Anatomy and School of P 6 OT, McGill U, Montreal, Canada, H3A 2R2. Dept of Physiology, U of Ottawa, Ottawa, Canada, K1H 8A4.

After unilateral hindlimb denervation of 2 wk mice for 4 wk or of 12 wk mice for 20 wk, sections of denervated (DN) and contralateral normal (NOR) extensor digitorum longus (EDL) and soleus (SOL) muscles were stained for ATPase (I, IIA and IIB fiber types) or immunohistochemically using monoclonal antibodies against fast (IIA or IIB) or slow (I) myosin heavy chain. Sections were examined for relative proportions of the various fiber types. Denervation had no effect on the total number of fibers but the proportions of fiber types were altered. Immunohistochemically, DN muscles of young mice exhibited complete loss of type I fibers, whereas the proportion of those in muscles of older mice were unaffected; for all mice there was marked de-differentiation of type II fibers in DN EDL and SOL. Immunohistochecmistry indicated very different results: fibers containing type I myosin increased in both DN EDL and SOL; in the latter they comprised nearly 100% compared to 33% in NOR controls. In DN EDL there were more fibers containing IIA and fewer with IIB myosin than NOR. These results indicate that histochemical and immunohistochemical techniques of fiber classification are not equivalent when examining plastic changes resulting from a perturbation such as denervation. Support: MRC and MDAC.
495.5  GENDER SPECIFIC MUSCLE FIBER AND STRENGTH ADAPTATIONS IN THE HUMAN BICEPS BRACHII.
G. E. Alway, J. Staley-Gunderson, W. J. Covey and W. M. Grumbach. Department of Cell Biology and Anatomy, UT Southwestern Medical Center, Dallas, TX 75235.

Isokinetic strength measures and computerized images of muscle cross-sections were obtained from 7 male and 5 female bodybuilders (BM) and 5 male and 5 female controls (NC). By analyzing single linear relationship between muscle CSA and strength and relates a new criterion for strength of both flexion and abduction muscles. The CSA of the muscles was determined in SA, St, and TEN and compared to similar data obtained in independent of the normal size-histochemical relationships. The proportion of VSMFs in depleted fast twitch muscle fibers (VSMFs) in TEN (Lev-Tov et al. J. Neurophysiol. 59: 1988) reflect an architectural feature in parallel-fibered muscles that is significantly different in the areas of their smallest fibers. Thus far, VSMFs appear to represent a major architectural feature in parallel-fibered muscles that is independent of the normal size-histochemical relationships.


The contractile properties of individual motor units of human thenar muscles were examined by a new method derived from the technique of microneurography and analogous to that used in animal studies. Forces or both flexion and abduction muscles were recorded simultaneously from the thumbs. In both the proximal and distal muscle surfaces, a tungsten microelecrode was used to stimulate single motor axons in the median nerve above the elbow. When the stimulus current was increased from zero, unitary activity was accepted if: a) no force or EMG responses were seen below a critical stimulus intensity; b) signals then appeared simultaneously, and remained unchanged over a wide range, usually 2-4 μA, before any graded increments appeared; c) X-ray photographs of the abduction and flexion forces showed a characteristic force vector for each unit. These criteria were satisfied repeatedly for 45 units from 12 subjects. Unit responses often remained stable for up to 1 hr while applying different stimulation protocols, including 1 s bursts of constant frequency (1-100 Hz), pulse intervals varied to optimize force generation, and standard fatigue tests. Respiratory and circulatory baseline fluctuations were minimized by triggering each stimulus packet from the heart beat, and electronically re-setting the baseline to zero just prior to each response. Contractile speeds, force / frequency curves and axon conduction velocities were measured before and after fatigue. Burke fatigue indices were calculated, together with each unit’s characteristic angle of pull within the muscle.

Supported by USPHS and the Swedish Medical Research Council.
495.11
AN ANATOMICAL STUDY OF MOTOR END PLATES IN A COMPARTMENTALIZED MUSCLE: THE CAUDAL R 그러면, Dept. of Zoological Sciences, Florida International University, Miami, FL 33139
Previous studies (English & Letbetter '82, English & Weeks '84, Weeks & English '85, '87) have suggested that neuromuscular compartmentalization is a basic organizational feature in some skeletal muscles. To determine whether neuromuscular compartments can be reestablished following injury, the mouse LG muscle has been adopted as a study model. A baseline for compartmentalization in this muscle is presented. The present study analyses the quantitative and qualitative profile of motor end plates (MEPs) in the mouse LG muscle using acetylcholinesterase, bromoindigo and silver, or zinco-iodide osmium staining methods. Results show that MEPs are distributed on equatorial zones of both superficial and deep muscle fibers in each compartment. Type b c MEPs (classification of Kornelussen & Wehrhae '73) are found throughout each compartment. Type b c MEPs tend to be more commonly found in distal and ventral compartments which consist of more oxidative muscle fibers. Additionally, MEPs were also observed in various stages of degeneration. The frequency with which degeneration occurred tended to be associated with collateral rebranching and did not appear to be compartment specific. This definition of the basic anatomy of MEPs is an attempt to further characterize LG neuromuscular compartments and make it more convenient for studying compartmental reinnervation.

495.12
ARCHITECTURE AND PERIPHERAL INNERRATION OF A MULTI-SERPENTINE MUSCLE IN THE PTEROPOD MOLLUSC CLIONE LIMACINA. Z. Huang* , D.S. Weeks & C.L. MacKenzie. Department of Zoology, Arizona State Univ., Tempe, AZ 85287. Tactile stimulation of the wing-like parapodia of the pteropod mollusc Clione limacina can trigger a wing retraction reflex, during which the wing is deflated and pulled into the body. Full retraction is achieved within 2-3 seconds and the wings usually remain retracted for 20-40 seconds. The magnitude of retraction is a graded function of the stimulus intensity. Three groups of smooth muscles involved in wing retraction are found in the wing haemocoele: the transverse muscles, longitudinal muscles and dorsoventral muscles. Furthermore, two subtypes of muscle cells were identified. The first type (type A) appears in all three groups of muscles and forms a well organized lattice-like structure. The second type (type B), being the major component of transverse muscles, run only one dimensionally. Quantitative ultrastructural comparisons between the two types of smooth muscle cells suggest the type A cells are able to contract and relax more quickly with low endurance while type B cells are capable of generating stronger contractions with higher endurance and slower relaxation speed. The role of these cell types in wing deflation and retraction is now under investigation.
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT VIII

TRANSITION OF PHYSIOLOGICAL TREMOR OF THE FINGER INTO FATIGUE TREMOR. S.G. Palmeter and S.J. Jackson. Dept. of Physiology, McGill University, Montréal, Québec, Canada.

To resolve conflicting reports from different laboratories in regard to the transition of tremor frequencies and amplitude, we measured tremor-induced submaximal contractions, human subjects (6 female, 6 male) were asked to elevate the middle finger of their nondominant hand 2 cm above the table and hold it steady for 45-60 min. A lightweight accelerometer was taped to the finger to record tremor. ENG was recorded from extensor digitorum, data stored on a tape recorder was analyzed using a frequency analysis program and an IBM PC/AT. Stretch reflexes were tested before and after the task by averaging 20 trials of flexed and extended digits. All subjects showed tremor frequencies between 15-45 min, ranging from 2-115 (N=28) times the original amplitude. Three subjects showed a progressive decrease from 15-33 min, but even in these subjects variability predominated over clear progression. In 10 subjects, tremor decreased after stretch were decreased in amplitude after the task by 18-76% (N=16). 7/12 subjects showed a sudden onset of a 4-6 Hz tremor in phase with the 4Hz finger tremor. All humans probably experience greater physiological tremor with fatigue, but their susceptibility to a 4-6 Hz tremor varies.
The sequential coordination of eye, head and hand movement is well studied under task conditions (e.g. target-oriented movements). In natural behavior, there is an initial period of sequential grouping to eventually grasp a stationary object. As the infant gains sufficient accuracy, grasping is typically optimized to one reach. How does the brain learn about the mistakes and experience that in turn incorporate it into accurate control of grasping that is generalized throughout the volume of space? I will show an implemented neural model that suggests an answer.

Studies by Held, Hein and others in the last two decades have shown that, in the kitten, visually guided behavior develops only when changes in visual stimulation are systematically related to self-produced movement. This work extends these results by hypothesizing that a sequence of key changes establish an intersensory correlation between object sensation and object manipulation.

The general strategy for accommodating behavior for space is the sensory-motor neural circular reaction. In this reaction, motor activity for the entire range of grasping postures or of an object is accomplished by some activator source. During each posture the two eyes see the 2-D projection of the 3-D object. Visual map activity is then correlated with whatever motor map activity was used to grasp the object. The correlation occurs from the changes of synaptic weights between visual inputs and motor outputs. After the correlation is learned, any object that is seen can trigger, as visual maps to activate the motor map for the intended grasping posture of that object.

There are two key aspects to the current model called INVANT (Interacting Networks Functioning on Adaptive Neural Topographies): 1) A distributed, topographic architecture is used to combine the visual activity from, both eyes and interface it with motor outputs. 2) Learning is achieved by incrementally modifying the distributions of input weights to the target map over single or sequential performance trials. On any given trial, only the weights of the active inputs in the trial are changed.

The neural model has been implemented into a working robot system with stereo cameras. The implementation learns to accurately grasp an object anywhere in space with one reach from the experience of sequential grouping.

The distribution of EMG activity of different neck muscles in relation with orienting head movements has already been studied in the alert trained cat (Roucoux, A. et al. Soc. Neurosci. Abst., vol. 19, p. 591). It has been shown, that on basis of the intensity of its global discharge, a preferential orientation could be attributed to each muscle. However, control over a relatively wide angle with the consequence that many muscles contribute to a given movement. The purpose of this study was to extract the individual contributions of the different muscles as a function of the direction of the head movement. The latency, i.e. the delay between the burst and movement onset, was greatly influenced as the movement direction diverges from the preferential orientation of the muscle: from a negative value of about 60° (the burst precedes the movement) to a positive 70° (the burst occurs during the movement). Some muscles exhibit late activity, occurring around the midcourse of downward movements. They may show inhibitions whose latency and duration are related to the direction of the movement.

In conclusion, the head motor system controls the direction and amplitude parameters not only by selectively activating the appropriate muscles but also by sequencing their activity to start, control the trajectory and stop the movement.

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In conclusion, the head motor system controls the direction and amplitude parameters not only by selectively activating the appropriate muscles but also by sequencing their activity to start, control the trajectory and stop the movement.
TOPOGRAPHIC MAPPING OF PRESACRAL BRAIN ELECTRICAL ACTIVITY IN HUMANS: SUPPORT FOR THE DUAL PREMOTOR SYSTEMS HYPOTHESIS. G. Goldberg and M. Moser*. Electrodiagnostic Center, Moss Rehabilitation Hospital and Temple University School of Medicine, Philadelphia, PA 19141.

Voluntary saccadic eye movements are preceded by a series of event-related potential (ERP) components. The presaccadic negativity (PNS) beginning 500 to 800 ms before the saccade-onset is reliably recorded in advance of the saccade and may reflect activation of the supplementary motor area (SMA). Decrease of the PNS latency by a brief, large amplitude component called the spike potential (SP) immediately precedes the saccade. Cortical unit and microstimulation in the superior colliculus (S III) in cynomolgus (Macaca fascicularis) and rhesus monkeys (Macaca mulatta) has documented the presaccadic potential (PSP) as a presaccadic related intermediate region in SMA to the dorsomedial surface of the frontal lobe. (1987) and regional cerebral blood flow (CBF) studies in humans (For PJ Fisk et al. 1983).

A presaccadic peak in the saccade-related intermediate region neurotoxic at SMA to the dorsomedial surface of the frontal lobe. (1987) and regional cerebral blood flow (CBF) studies in humans (For PJ Fisk et al. 1983).

The dependence of the topography of presaccadic ERPs on behavioral context was examined in six normal human subjects. The degree to which timing and direction of saccades were environmentally constrained by visual information was systematically varied. Our general findings are as follows: (1) Prior to self-initiated “voluntary” saccades generated in the dark, there is a large, vertex-centered PNS which may be related to preparatory activation of the SF. (2) The PNS is greatly attenuated when the timing and direction of saccades are commanded by visual stimulus. (3) The SP demonstrates a large negative focus over the lateral frontal electrode in the direction of the saccade suggesting that it is probably related to EMG activity from the underlying lateral rectus muscle (Thickman GW & Massigla FL. Brain Res 339:271-280, 1985). (4) this method provides dynamic information which complements that of cortical unit recording in subhuman primes and CBF recording in humans using PET. These findings are generally consistent with the idea that the SF may play a special role in self-initiated, endogenous saccades and provide support for the dual premotor systems hypothesis as applied to the generation of voluntary saccades.
**MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT VIII**


The present work characterizes the activity patterns of single muscle units during spontaneous rhythmic jaw movements (RJM) in ketamine-anesthetized rats. Unit recordings were obtained from the dorsal and ventral histochromatographic compartments of the anterior digastric muscle using teflon coated stainless steel wires (uncoted diameters 90-150 µ).

The analysis of 5 different units (in 6 preparations) revealed 3 different patterns of activity: 1. continuous tonic activity at slow and regular firing rate (mean=25 Hz). 2. repetitive bursts of various duration at a slow and regular firing rate. 3. high frequency (mean=65 Hz) rhythmic activity synchronized with the EMG bursts during RJM. The first two types of activity were observed mainly in the ventral compartment which is characterized by the absence of slow-burst muscle units (Lev-Tov, A. and Tel., M., J. Neurophysiol. 59:496, 1987). The third type was evident in both compartments during the rhythmic EMG bursts and is likely to involve recruitment of fast-out of motor units.

Further studies using refined recording techniques are now under progress.

496.22 COMPARISON OF SINGLE MUSCLE ACTIVITY PROPERTIES IN PERIODICALLY GRAND AND NUCLEUS RETROAMBIGUOS DURING VOCALIZATION IN MONKEYS. E.A. DeRosier*, R.A. West*, C.R. Larson. Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208.

Previous studies have shown that the midbrain periaqueductal grey (PAG) is involved in vocalization, but its precise functional role is not yet understood. An understanding of the localization of vocalization-related PAG neuronal projections. Hohagen (1987) demonstrated that PAG neurons project to the nucleus retroambiguus (NRA) and that PAG and NRA cells project to the ventral horn of the spinal cord and the nucleus ambiguus, suggesting that the NRA may be involved in vocalization.

To test the hypothesis that NRA cells are involved in vocalization, extracellular recordings of NRA neurons were made in Macaca nemestrina monkeys trained to vocalize. Simultaneous recordings were made of the laryngeal and respiratory muscle activity and vocalization. Activity of the NRA neurons was evaluated with respect to vocalization and muscle activity and compared with PAG neuronal activity recorded under similar conditions.

In and around the NRA, cells with a variety of discharge patterns were observed, and some of these patterns were similar to those observed in the PAG. A few NRA cells were inactive and became active just before and during vocalization. Many cells displayed a respiratory rhythm and discharged either in phase or out of phase with one or more of the respiratory muscles. Other neurons were phasically active with oral-facial movements and may be related to lingual, masticatory, facial, palatal or pharyngeal muscle activity. Cross-correlations between units and EMGs indicated that NRA cells were more highly correlated with vocalization muscles than PAG cells. Microstimulation at PAG recording sites excited muscles with latencies of 12-20 ms. Microstimulation in the NRA excited muscles at 5-10 ms latencies. The data suggests that the idea that cells in and around the NRA are involved in vocalization. The NRA may serve as a relay and integration site for descending inputs to laryngeal and respiratory motorneurons.

497.1 CELLULAR PROPERTIES OF PURKINJE CELLS AND EXCITATORY NEURON DISCHARGE IN TRANSLUMINATED TURTLE CEREBELLM. T. Staele, D.R. McCrimmon and J.F. Larson. Prior. Dept. of Physiol., Northwestern University Medical School, Chicago, IL 60611 USA.

The intact turtle cerebellum can be maintained in vitro for several days, allowing long-lasting (1-10 hr) somatic and dendritic intracellular impalements of Purkinje cells (PCs). We have compared the electrophysiologic and pharmacologic properties with mammalian PCs. The data indicate that Purkinje cells in the turtle cerebellum share many common properties of Purkinje cells in this preparation and the responses of impalements of Purkinje cells (PCs). We have studied the electrical properties of Purkinje cells in these preparations, revealing 3 different patterns of discharge: 1. Continuous tonic activity at slow and regular firing rate (mean=25 Hz). 2. Repetitive bursts of various duration at a slow and regular firing rate. 3. High frequency (mean=65 Hz) rhythmic activity synchronized with the EMG bursts during RJM.

The first two types of activity were observed mainly in the ventral compartment which is characterized by the absence of slow-burst muscle units (Lev-Tov, A. and Tel., M., J. Neurophysiol. 59:496, 1987). The third type was evident in both compartments during the rhythmic EMG bursts and is likely to involve recruitment of fast-twitch motor units.

Further studies using refined recording techniques are now under progress.


A survey of brain glucose utilization (GU) was carried out to identify brain regions showing abnormal metabolic rates in the genetically dystonic (dt) rat, a mutant with a movement disorder involving twisting of the limbs and axial musculature. For 19 rats 19-24 days old, GU was estimated with arterial plasma data (Sokoloff, 1977). Animals were quieter during the experimental period. All 6 dt rats and 5 of 8 unaffected littermates had an overall GU rate lower than that of 5 normal non-littermates in the 61 regions analyzed (Mean difference, -37%). The metabolic rates were significantly lower than controls in the deep cerebellar nucleus (e.g. dentate, p<0.01). A z-score analysis was used, differences were found in the deep cerebellar nuclei, locus coeruleus, pontine gray, red nucleus, principal nucleus of the third nerve, ventrolateral and ventromedial nuclei of the thalamus, lateral habenula and basolateral nucleus of the amygdala. The data suggest that the cerebellum and its efferents are the focus of abnormal neural activity in this mutant. (Supported by NS10626).

497.3 GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY AT CEREBELLAR PROJECTION FRAMES IN THE DYSTONIC RATS. M. Oitmans, M. Beal* and J.F. Lorden. Dept. of Pharmacology, Med. School, Chicago, IL 60664 and Dept. of Psychology, Univ. of Alabama, Birmingham, AL 35294.

Recent work has revealed the presence of a GABAergic projection from the deep cerebellar nuclei (DCN) to the inferior olive (10) (Nelson, et al, 1984, Soc. Neurosci. Meeting). The intact turtle cerebellum can be maintained in vitro for several days, allowing long-lasting (1-10 hr) somatic and dendritic intracellular impalements of Purkinje cells (PCs). We have compared the electrophysiologic and pharmacologic properties with mammalian PCs. The data indicate that Purkinje cells in the turtle cerebellum share many common properties of Purkinje cells in this preparation and the responses of impalements of Purkinje cells (PCs). We have studied the electrical properties of Purkinje cells in these preparations, revealing 3 different patterns of discharge: 1. Continuous tonic activity at slow and regular firing rate (mean=25 Hz). 2. Repetitive bursts of various duration at a slow and regular firing rate. 3. High frequency (mean=65 Hz) rhythmic activity synchronized with the EMG bursts during RJM.

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Further studies using refined recording techniques are now under progress.

497.4 QUANTITATION OF CEREBELLAR CELL NUMBER IN REELER MUTANT MICE. B. Beckett*, J.A. D. Goldowitz, and L.M. Eiseman, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107. The reeler mutant mouse suffers from a serious genetically imposed deficit in nervous system histogenesis, believed to be based on a generalized disruption of neuronal migratory mechanisms. The Purkinje cells of the reeler cerebellum are located in abnormal aggregates, instead of in their normal superficial monolayer. Their abnormal position, and possible mixing with other cell types has precluded any study of their number in this mutant. We have used antiserum to the specific Purkinje cell marker, CCP-dependent protein kinase (gift of F. Greenfield) to identify PCs in the reeler, and to thus quantify their number. Our results indicate that reeler mice have an average of 8,500 Purkinje nuclei, 15% reduction from the normal number of 10,000 cells. This apparent reduction is in line with studies of other mutant lines which seriously effect Purkinje cell number where little loss of cerebellar nuclear cells is observed.
497.5 c-fos EXPRESSION AND 14C 2-DEOXYGLUCOSE UPTAKE IN THE CEREBELLUM: DIRECTIONAL MOTOR / SENSORI MOTOR INTEGRATION IN THE RAT. Frank R. Sharp, Manuel F. Gonzalez, Stephen N. Sagat, James V. Sharp, Tom Curran*, and Kathleen R. Zahs. Departments of Neurology and Physiology, University of California at San Francisco and VA Medical Center, SF, CA 94121.

Electrode stimulation of hindlimb motor / sensory cortex of awake rats increased 14C 2-deoxyglucose (2DG) uptake diffusely as well as in granule cell patches in the dorsal and ventral lamellae of the eighth cerebellar hemispherical lobule, copula pyramids (CP). Forelimb cortex stimulation and trained forelimb movements activated the same regions of the paramedian lobule and copula pyramids (CP). c-fos gene expression was examined using Fos protein immunocytochemistry 3 hours following a fifteen minute period of hindlimb cortex stimulation. Induction of Fos occurred in similar granule cell patches as those seen with 2DG. Fos was also induced in discrete Purkinje cell patches. Activated Purkinje and granule cell patches either wholly overlapped, partially overlapped, or were non-overlapping at various points along the CP lobule. The results support prior suggestions of compartmentalized processing of mossy fiber inputs and Purkinje cell outputs, and show that granule and Purkinje cell patches may be non-congruent as well as congruent.

497.7 DIFFERENT INFORMATION IS ENCODED BY SETS OF CLIMBING FIBER RECEPTIVE FIELD MAPS IN THE ANTERIOR LOBE VERSUS THE PARAMEDIAN LOBULE. L.T. Robertson and C. McCollum. Robert Dow Neurological Sciences Institute, Portland, OR 97209-1595

The composition of climbing fiber receptive fields is related to the type of information encoded and to the ensembles of cells that participate in the encoding. An analysis was made of receptive fields of the face and the distal paw from individual climbing fiber responses encountered in the anterior lateral line nerve (AL) and paramedian lobule (PML) of the anesthetized cat. The boundaries of the receptive fields of the face or distal paw form compartments of responses recorded in both regions are unions of compartments. Analysis of the inclusions of various compartments for the paw reveals that the lateral parts of the forepaw and hindpaw are included in a large proportion of the receptive fields for cells encountered in the PML, whereas the medial parts of the paw are more frequently included in forepaw receptive fields of cells located in the AL. Although many of the receptive fields are identified in both cortical regions, the proportion of the various compartments represented in the two regions are different. Thus, mechanical stimulation of certain parts of the paw or face will synchronously elicit different ensembles of climbing fiber responses in the AL versus the PML.

497.8 RESPONSE OF RAT PARAFOCULAR NEURONS TO COMBINATIONS OF VISUAL AND AUDITORY STIMULI. Brian N. Maddow, S.A. Aziz, and P.J. Wood. Eye Research Laboratory, Dept of Cell Biology and Vision Research, UT Southwestern Medical Center, Dallas, Texas 75235.

As part of an ongoing investigation into sensorimotor integration in the cerebellum, previous reports from this laboratory have demonstrated visual and auditory inputs to the rat paraflocus which have shown that these projections arrive via the dorsolateral basilar pontine nuclei. We now report that combined auditory and visual stimuli in the unanesthetized preparation. In this study, recordings from paraflocus neurons were obtained with glass micropipettes in immobilized, locally anesthetized Long-Evans rats during visual and auditory stimulation. Selected images were projected onto a screen in front of the rat, while orientation, position, and velocity of travel were controlled by a computer. Delivery of a tone was also controlled by the computer, such that it was possible to deliver a variety of visual and auditory stimuli, each given alone or in combination with the other modality. Temporal offsets between paired stimuli can be varied systematically. The general finding is that combined auditory and visual stimuli facilitate response of paraflocus neurons when compared to the response to single modality stimulus. Subthreshold inputs are clearly not "weak" inputs, since they are capable of powerful control over the dynamic range of firing when properly biased by input from a paired modality. Supported by the Biological Humanities Foundation

497.9 TRIGEMINAL NERVE SECTION IN NEONATES LEAVES HOPLES IN CEREBELLAR GRANULAR LAYER TACTILE MAPS OF ADULTS. Ted Milner & Allan M. Smith. Univ. of Montreal, Quebec, H3C 3J7.

The granular layer of crura I and II of the rat cerebellar cortex contains a tractured somatotopic map of perioral tactile receptive fields (fista, vibrissae, teeth etc). Because non-rotating, sensory input to this region represents the r Norton (1971) have described the somatotopic representation of the trigeminal nerve in 9-day postnatal rats causes Crus I, regions which contain tactile maps in cerebellar cortex is established inflexibly during development. (Supported by NIH grant 52205 and the Lucille P. Markey Foundation).

497.10 CEREBELLAR UNIT ACTIVITY DURING POSTURE AND MOVEMENT OF THE WRIST AGAINST CONSTANT TORQUE. Ted Milner & Allan M. Smith. Univ. of Montreal, Quebec, H3C 3J7.

A monkey was trained to move a wrist manipulandum to 3 target zones and then to maintain the position. We recorded wrist position, accelerations, torque direction, EMG, extensor EMG and discharge of single cerebellar units while the monkey was performing 1) movement that a constant flexor or extensor torque, 2) holding a target position against a constant flexor or extensor torque.

Conditions 1 and 2 elicited a reciprocal pattern of muscle activation from the prime movers of the wrist. When the torque associated movement, activity in the agonist muscle gradually increased as movement progressed from the initial to the final position, while the torque assisted movement, activity in the antagonist muscle gradually decreased throughout the movement.

We have recorded from approximately 50 task-related units (granule and Purkinje cells) in wrist and hand regions of the cerebellar cortex. Although the discharge rate for many cells was higher during movement than holding, inhibition did occur in some cases. Discharge rates were also related to EMG, target position, torque direction and movement direction.

FRIDAY AM
MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM

Cerebellum plays an important role in adjusting observed/anticipatory responses & compensatory changes consisted of an increase in the EMG rate of grip force application. Compensatory changes consist of an increase in the DMG activity of muscles most directly related to gripping, at latencies between 100 to 300 ms and a subsequent increase in the grip force. From a population of neurons recorded in the paravermal hand area of the cerebellar cortex about 40% of the neurons showed compensatory increases in activity between 40-60 ms. Some cerebellar neurons responded to both anticipated and unexpected object slippage between the fingers. Supported by the Medical Research Council of Canada and the Fonds F.C.A.R. du Québec.

THEORETICAL PREDICTIONS OF SPATIAL ANOSTEROCITY OF ACEREBELLAR SYMMETRY IN HEAD MOVEMENTS OF CATS A.J. Pellionisz and B.W. Flanders. Cognitive Neurophysiology Laboratory, Good Samaritan Hospital, Portland, OR 97210. First, sensor network theory puts forward a concise explanation of the coordination-function of the massively parallel cerebellar neural networks: a geometrical tensor representation of two-dimensional intrinsic sensorimotor coordinate frames. Further tests of the validity of current tensorial models of CNS function in multidimensional intrinsic sensorimotor coordinate frames.
497.17 HISTORY OF STEROIDITY IN NEUROSCIENCE I: THE ORIGINS. L.H. Magoun and H.W. Magoun, National Institute of Mental Health Program, Brain Research Institute, University of California, Los Angeles, CA 90024.

After Hitzig and Fritsch in Berlin demonstrated conclusively in 1870 that stimulation of points on the surface of the mammalian brain produced contraction of muscles on the opposite side of the body, investigations ex- periments were conducted in England. Responses form the cerebral surface of subhuman primates were mapped with a good deal of precision. Obtaining results from below the surface was neither precise nor reproducible. Victor Horsley, London's preeminent neurosurgeon, combined a thriving practice with laboratory experiments. He and Robert H. Clarke, a physician with engineering talent, attempted a study of function of the cerebellar nuclei. An improved technique was essential to control placement of the electrodes. Clarke applied geometric principles to the problem and designed and supervised the construction of the first instrument, which Horsley demonstrated in Canada at the annual meeting of the British Medical Association in 1906. The description of the Horsley-Clarke appeared in Brain two years later, but the experimental results were never published. The only important immediate use was by Ernest Sachs, a young American surgeon training with Horsley, who had a second instrument built and published his studies on the function and morphology of the optic thalamus. He then took the technique to America.

497.18 HISTORY OF STEROIDITY IN NEUROSCIENCE II: REVIVAL. H.W. Magoun and L.H. Magoun, National Institute of Mental Health Program, University of California, Los Angeles, CA 90024.

The experimental use of the Horsley-Clarke stereotaxic instrument, introduced in 1906, was largely forgotten for almost 20 years and then was revived through an unusual chain of events. Shortly before he died in 1926, Robert Clarke, the instrument's inventor, urged Ernest Sachs, a neurosurgeon at Washington University, St. Louis and owner of the second instrument constructed, to continue the studies on the cerebellum that Horsley and Clarke had begun in England. Sachs was conducting such studies during the short time that Stephen Walter Ranson and Joseph C. Hiney were in St. Louis and saw the instrument in use. In 1928 they returned to Northwestern University and a copy was made of the model published in Brain and the worldwide use of stereotaxy in probing the Brain from external landmarks was assured. Control of movement at several levels of the central nervous system was the first such exploration of so extensive a field. Before Ranson and Hiney died unexpectedly in 1942, the role of the hypothalamus and lower brain stem in visceral integration, emotion, and the regulation of feeding, fighting, sex, and other behaviors was elucidated. The instrument was adapted to many animals, including man, and the technique improved. However, 80 years after Clarke, the determination of internal cerebral loci by external landmarks has been made obsolete by the new imaging techniques.

EFFECTS OF CHRONIC DRUGS


Prenatal haloperidol (HAL) exposure in rats is known to reduce striatal D2 receptor binding sites in postnatal day 30 offspring HAL exposure on striatal D1 binding sites, and D1 and D2 binding sites in other regions of the dopamine (DA) system was not well described. However, CD CD rats were given daily injections of HAL (2.5 or 5 mg/kg, s.c.) vehicle over gestational days 6-20. D1 and D2 DA receptor binding was measured in striatum, accumbens, amygdala and frontal cortex of male and female offspring sacrificed on postnatal day 30. In HAL treated animals there was a 20% decrease in both D1 and D2 and binding sites in striatum, with a similar reduction in D2 but not D1 sites in accumbens. There was no HAL effect on D1 or D2 binding in amygdala. The data indicate that prenatal HAL exposure results in DA receptor binding that are specific to brain region and DA receptor subtype. The results further suggest that the developing nigrostriatal DA system may be more at risk for prenatal pharmacological insult than is the mesolimbic DA system.


Chronic prenatal haloperidol (HAL) treatment has been shown to have pronounced behavioral, psychopharmacological and neurochemical effects. Preganglionic denervation (DA) DA autoreceptor function was assessed in the DA terminal rich regions nucleus accumbens, caudate nucleus and olfactory tubercles of rats. HAL treatment was used to decrease the levels of 3,4-dihydroxyphenylalanine (DOPA) and DA accumulation by HPLC following administration of the DOPA decarboxylase inhibitor NSD-1015. Probenecid, which is known to block urine or bile excretion of newly synthesized substrate, was used to maintain the transplacentally administered DOPA as an extracellular substrate in HAL-treated animals. 

498.3 EFFECT OF INJECTION SCHEDULE ON TOLERANCE TO HALOPERIDOL-INDUCED 'ANOREXIA.' D.L. Holman, Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

We previously found that rats given daily injections of haloperidol became tolerant to the initial 'anorectic' effect whether the drug was given before or after access to food. These results suggest that tolerance is not contingent on access to food in the drugged state. However, because the drug is long-acting, it is possible that the drug after access was actually intoxicated during testing. To control for this possibility, rats in the present study were given the drug following access to milk on alternate days. Control rats were given injections of saline. On the intervening days, all groups received the critical basal levels of intake. Contrary to previous findings, little tolerance was observed in either drug group on test days, although baseline intake was consistent. These results suggest that injection schedule plays an important role in tolerance to haloperidol-induced 'anorexia.'

498.4 DECREASED STRIATAL(S) ACH RELEASE FOLLOWING CHRONIC HALOPERIDOL(H) TREATMENT. R.E. Peck, M.B. Hank and P. Butkereit* Medical College of Pennsylvania, Philadelphia, PA 19129.

The effect of chronic H treatment on [3H]ACH release from superfused S slices was assessed. While acute and chronic H produced increases in [3H]ACH release, following chronic treatment produced decreases (34-38%) in evoked [3H]ACH release. SKF-38393 produced dose-dependent (0.1-10uM) increases in [3H]ACH release which were blocked by SCH 23390 and by bicuculline. The effect of D1-receptor stimulation was significantly reduced after 2.5 and 5 mo of H treatment. Both L-7171555 and carbachol produced dose-dependent increases in [3H]ACH release. Long-term treatment with H (2.5 and 5 months) elicited increased sensitivity to the effect of L- 7171555 while the effect of carbachol was diminished following a 5 mo treatment period. The apparent desensitization of presynaptic muscarinic receptors coincided with a 10% decrease in the number of muscarinic receptors labelled by [3H]-methylquinine but not of those labelled by [3H]pirenzepine. These findings demonstrate that withdrawal from chronic H produces a hypolaminergic state in the striatum. These findings suggest that diminished S ACh release observed following discontinuation of long term H treatment may contribute to the emergence of tardive dyskinesia.
EFFECTS OF CHRONIC DRUGS

498.5

Chronic treatment of rats for 6-12 months with neuroleptics has been proposed as an animal model of tardive dyskinesia. We have examined this paradigm by studying haloperidol (HAL)-induced vacuous chewing movements (VCM) in the rat for 6 weeks. 38 male rats were treated, comprised of 3 strains: Sprague-Dawley (SD), Long Evans (LE), and Wistar (W). Nine rats of each strain were treated with 1.5 mg/kg/day of HAL orally through their drinking water; four (3W) rats were water control, with the exception of neurotensin which was dropped 50% during withdrawal. Performance on FR was unchanged by the amount of training per se, and rather continued training during chronic treatment provides the opportunity for reinforced responding to shifting drug and nondrug cue states.

498.7

The effect of long-term haloperidol administration on altered behavior and the development of tolerance. Rats were trained to discriminate amphetamine in a two-lever drug discrimination task. Two groups were then given a chronic drug regimen of 13 daily injections of either distilled water or 10 mg/kg amphetamine. Drug discrimination training was continued for half of each chronic drug group. Tolerance was observed only for the group that was not trained during the chronic amphetamine treatment. The data show that (a) choice responding after chronic drug treatment is not influenced by the amount of training per se, and (b) rather continued training during chronic treatment provides the opportunity for reinforced responding to shifting drug and nondrug cue states.

498.8

Psychoactive drugs produce distinct primary and compensatory adaptive processes that occur in temporal sequence. These adaptive processes are thought to be the basis for pharmacodynamic interaction. The purpose of this experiment was to evaluate the extent to which chronic drug discrimination training during chronic treatment effects the development of tolerance. Rats were trained to discriminate distilled water from 0.75 mg/kg amphetamine in a two-lever drug discrimination task. Two groups were then given a chronic drug regimen of 13 daily injections of either distilled water or 10 mg/kg amphetamine. Drug discrimination training was continued for half of each chronic drug group. Tolerance was observed only for the group that was not trained during the chronic amphetamine treatment. The data show that (a) choice responding after chronic drug treatment is not influenced by the amount of training per se, and (b) rather continued training during chronic treatment provides the opportunity for reinforced responding to shifting drug and nondrug cue states.

498.9
EFFECT OF ANTIDEPRESSANT DRUGS ON SURVIVAL OF CEREBRAL NEURONAL HOMOLOGUES IN THE DEVELOPMENTAL STAGE. C. G. Wogelser, J. Banner, A. Flatman, W. M. Aldrich. University of Pennsylvania School of Medicine, Philadelphia, PA, 19104.

Repeated administration of many antidepressants to rats reduces the density of beta adrenergic receptors (BAR) in the cerebral cortex, as revealed using ligand binding techniques on homogenate in all areas. The selectivity of the inhibitor of the uptake of serotonin, citalopram.

498.10

Amphetamine or cocaine withdrawal has often been reported to produce depression in humans. We investigated whether this happens in rats because of the possibility of subsequent neurochemical abnormalities. Because of the anhedonia in depressed humans, most of our tests focused on the effects of training and emotional status, which are altered in depression. The study demonstrated that there was a marked decrease in TD (average reduction of 43.5 ± 32.9%) in 17 patients during the neuroleptic free period. Three patients who did not show improvement were all elderly (>65 yrs) females. Multiple regression analysis revealed a significant negative correlation (partial r = -0.38, p<0.03) between the age of the patients and their observed change in TD score. There was also a significant correlation between the duration of TD free period and the change in TD scores (partial r = 0.41, p<0.03).

These results strongly suggest that TD is reversible, especially in young patients. Further, they emphasize the utility of atypical neuroleptics (e.g. clozapine), which purportedly exert low or no DA receptor blockade in the basal ganglia, in the treatment of psychotic patients with TD.
EFFECTS OF CHRONIC DRUGS
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498.11 EFFECTS OF CHRONIC LITHIUM TREATMENT ON RECEPTOR-MEDIATED INOSITOL PHOSPHORYLATION IN RAT BRAIN. T. Casebolt* and R.S. Jope, (SPON: A.L. Beckman), Dept. of Pharmacology, Univ. of Alabama, Birmingham, AL 35294

The etiologies of mania and depression, and the mechanism of action of lithium as a treatment for these disorders, are unknown. To test the hypothesis that a part of the therapeutic effect of lithium is due to altered receptor-coupled phosphoinositide (PI) hydrolysis, rats were treated with LiCl (0.17% in diet, 30 days) and agonist-induced PI hydrolysis was measured in rat brain. Neuripilin, but not carbachol-induced PI hydrolysis was significantly reduced by chronic lithium treatment. Experiments examining the mechanism of this effect led to the hypothesis that receptor number or receptor-coupling was reduced by chronic lithium treatment. Examination of the effects of chronic lithium treatment on α-adrenergic binding of [3H]prazosin and chlorpheniramine indicated that α-adrenergic receptor subtypes were not altered by chronic lithium treatment.

The possible role of protein phosphorylation in receptor uncoupling after chronic lithium treatment was also examined. Increased PKC activity in membrane and cytosol fractions was measured. Endogenous protein phosphorylation mediated by three kinases was also surveyed after chronic lithium treatment. Supported by MH 38752.


Intracerebral infusion of naloxone has been used to precipitate withdrawal in morphine-dependent rats. The present results indicate that the nigrostriatal dopaminergic system is involved in the development of physical dependence to this drug. Male Sprague-Dawley rats received subcutaneous (sc) injections of morphine sulfate by two intracerebral (nigral) preparations (Collier, et al., Nature, 233, 1972, 220) according to the following schedule: 40 mg/kg (day 1); 60 mg/kg (day 2); 80 mg/kg (day 3); and 100 mg/kg (day 4). Twenty hours after the last sc injection animals received a bilateral intranigral injection of naloxone (1-10 µg; 0.25 µl saline) or saline (0.25 µl). Intranigral injection of naloxone produced several significant (p<0.05) signs of withdrawal in morphine-dependent animals including wet dog shakes, teeth chattering, inappetence, and diarrhea. No withdrawal signs were observed in morphine-dependent animals that received intracerebral saline or in nondependent controls that received intranigral naloxone. There was a significant correlation (p<0.01) between naloxone dose and magnitude of the response for each withdrawal symptom. These results suggest that the SN mediates the development of physical dependence produced by repeated administration of morphine. (Supported by USPHS grant HD-21560).


We have examined the effects of naloxone-induced opioid receptor upregulation (two 30 mg pellets up to 9 days) on lateral hypothalamic self-stimulation (SS), stimulation-induced feeding (SIF), and stimulation-induced escape (SIE). Opioid receptor supersensitivity following naloxone withdrawal potentiated SS while SIF and SIE were unaffected. The observed increase in SS was reversed by acute injection of naloxone, demonstrating opioid receptor mediation. Upregulation-induced potentiation of SS occurred only in electrodes that did not support SIE. These were posterior to placements supporting SIE, as previously observed (S. Carlen and E. Coons, 1987).

While receptor upregulation increased sensitivity to opioids it has been shown previously (M. T. Bardo et al., 1983, B. C. Yoburn et al., 1985), this is the first report we are aware of that demonstrates supersensitivity to endogenous opioids. Furthermore, though past research has shown that opioids can modulate SS (K. D. Carr and E. J. Simon, 1984), the present results indicate that under conditions of supersensitivity opioids maintain SS as well. Paradoxically, while SIF is mediated by opioids under baseline conditions (K. D. Carr and E. J. Simon, 1983), it is unaffected by upregulation.


Caffeine was administered in three doses (10 mg/kg/day, 25 mg/kg/day and 50 mg/kg/day) to rats as twice-daily i.p. injections for 30 days. Plasma caffeine concentrations and regional brain concentrations of monoamines (dopamine (DA), norepinephrine (NE) and serotonin (5HT)) and their metabolites [dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-methoxy-4-hydroxyphenylglycol (MHPG)] were determined. In striatum, data was obtained for all the above except MHPG. In frontal cortex and cerebellum, NE, 5HT and their metabolites were determined. In addition, for each monoamine an index of turnover was calculated (metabolite/s/monoamine).

A linear dose-response relationship was found for plasma caffeine concentrations. At 10 mg/kg/day no monoamine changes were found except for a decrease in cerebellar NE. At the higher doses there were significant changes in all three brain areas. Striatal DOPAC decreased significantly, while DA increased (with a trend toward a lower DA turnover index). The 5HT index decreased significantly in striatum and cerebellum, with a similar trend in frontal cortex. The NE index decreased in frontal cortex, but increased in cerebellum (the only increase observed in the turnover indices).

498.15 EFFECT OF ORAL CAFFEINE INGESTION, ON WHEELRUNNING IN RATS. D.E. Landem, T.A. Landem, and C.J. Meliska. Department of Psychology, Southern Illinois University, Carbondale, IL 62901-6502.

Twenty-four male Sprague-Dawley rats were housed continuously for 24 days in Wahman activity wheels. Eight rats received continuous access to 0.5 mg/ml caffeine base in tap water; 8 received continuous access to tap water only; and 8 were deprived of 24 hr access to caffeine alternating with 24 hr access to tap water. Mean wheel revolution data for the caffeine/water (C/W) group revealed a significant decrease in water on day 2 compared to controls receiving continuous water, suggesting a withdrawal reaction to the absence of caffeine. When the data were converted to percentages of baseline wheelrunning, continuous access to caffeine failed to stimulate, and actually inhibited wheelrunning. Twenty rats were divided into two groups and placed pairs of rats alternating between caffeine and water (C/W), wheelrunning was significantly greater on caffeine days than for the other conditions tested in caffeine access. These data suggest that when given orally, caffeine's stimulatory effects on wheelrunning are particularly sensitive to the context and scheduling of drug administration.

498.16 CONTINGENT INEFFECTIVITY AND TOLERANCE TO CARBAMAZEPINE IN ANIMALS. H.R. Weis and D.J. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The relationship of the timing of drug administration to anticonvulsant efficacy against amygdala-kindled seizures was studied. During kindling development, rats received either no treatment or carbamazepine (15 mg/kg) before (carba-before) or after each stimulation (carba-after). After kindling to full seizures, all animals received carbamazepine before the stimulation. Both the drug-naive and drug-after group showed a good anticonvulsant response. The rats that received carba-before during kindling failed to develop tolerance to carbamazepine's anticonvulsant effects (conditioned ineffectiveness), which could be reversed by switching to carba-after or kindling the animals drug-free, but not by carbamazepine administration alone or after a time off from both treatments. Another group of rats were kindled with carba-after or vehicle-before to determine whether prior exposure to carbamazepine in a noncontingent fashion would facilitate the subsequent development of tolerance. When these rats were switched to carbamazepine treatment, both groups developed tolerance to carbamazepine at equal rates.

These data suggest that tolerance to or ineffectiveness of anticonvulsant medications may be learned phenomena susceptible to modulation through alterations in the temporal relationship between drug administration and seizure induction.
EFFECTS OF CHRONIC DRUGS TO THE CHICK: DEVELOPMENT OF MYOPIA

A mathematical model for emmetropization in the chicken. H.C. Howland and F. Schaeffle. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

Eye growth and refractive state in the chicken has been examined under a large number of experimental treatments. Degrading the retinal image by use of occluders produces myopia, although with a high variability in individual refractive errors. Image degradation of the myopic eye can be also produced in birds without accommodation (due to lesions in the Edinger-Westphal nucleus), with the optic nerve sectioned and, by local image degradation, in local parts of the visual field. Recovery occurs in both normal and Ewisioned chicks. In addition, we have shown that eye growth compensates for the loss of matrix proteins in the vitreous humor and the loss of the yolk sac. These results indicate that there are alterations in maternal behavior associated with cessation of MS administration. The presence of wet-dog shakes suggests that MS withdrawal contributes to these alterations. Future studies will assess the effects of this treatment regimen on neurotransmitter levels in other brain nuclei, supported by grant DA0181-01, M. D. Schechter, P.I.

CHRONIC DRUGS AND THE OTHER DEPENDENT ON ACCOMMODATION. SHOWED THAT THERE ARE PROBABLY (AT LEAST) TWO INDEPENDENT MECHANISMS WITHIN THE EYE, WITHOUT THE NECESSITY OF NORMAL AND MS-LESIONED CHICKS. MOST OF THE RESULTS LISTED ABOVE ARGUE FOR REGULATION OF EYE GROWTH BY LOCAL MECHANISMS WITHOUT THE NEED FOR NORMAL FUNCTIONALITY IN THE EYE.}

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY VI

THE VITREOUS HUMOR IN EXPERIMENTALLY INDUCED MYOPIA. B.L. Selwin and L. Weinstain. SPON: R. Beauchamp, School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ont. CANADA N2L 3G1.

Experimentally induced myopia can be produced rapidly in chicks subjected to post-hatching blurring of the retinal image. This myopia is characterized by axial and equatorial eye size increase, increase in wet weight of the eye, and a large negative refractive error, within 14 days of post-hatching exposure. Alterations in the behavioral development of the offspring associated with cessation of MS administration may contribute to the increased mortality and alterations in the behavioral development of the offspring.

IMPAIRMENTS RELATED TO LONG-TERM USE OF DRUGS IN CHRONIC PAIN PATIENTS. B. W. Kellogg*, E. L. Tune*, D. B. Pearson*, P. L. Walshe, P. H. Doryman*, & M. N. Rossel* Psychiatry Dept., Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cognitive use of benzodiazepines and opiates in chronic pain patients is poorly understood. Less is known about the influence of short-term fluctuations in drug level on behavior. Information about persistent effects of long-term use and fluctuations due to high vs. low levels may help to identify impediments to effective pain management. The purpose of the study is to determine neurophysiological and phenomenological correlates of naturally occurring variation in medication levels.

Injections on a chronic pain treatment unit were tested approximately 2 hrs post administration (peak) of benzodiazepines and or opiates and just prior to administration (trough) weekly for three weeks. The test battery included measures of psychomotor ability, distractability, memory, pain, and mood. Drug levels were measured via a saliva sample using radioisotope assay techniques.

Preliminary results show poorer performance at peak than trough levels in psychomotor speed, set shift and in learning-memory, although anxiety and pain levels did not vary significantly. The findings suggest that the short-term fluctuations in opiate/benzodiazepine levels may affect neurophysiological abilities contributing to functional incapacity in chronic pain patients.

A MATHEMATICAL MODEL FOR EMMETROPIZATION IN THE CHICKEN. H.C. Howland and F. Schaeffle. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

Eye growth and refractive state in the chicken has been examined under a large number of experimental treatments. Degrading the retinal image by use of occluders produces myopia, although with a high variability in individual refractive errors. Image degradation of the myopic eye can be also produced in birds without accommodation (due to lesions in the Edinger-Westphal nucleus), with the optic nerve sectioned and, by local image degradation, in local parts of the visual field. Recovery occurs in both normal and Ewisioned chicks. In addition, we have shown that eye growth compensates for the loss of matrix proteins in the vitreous humor and the loss of the yolk sac. These results indicate that there are alterations in maternal behavior associated with cessation of MS administration. The presence of wet-dog shakes suggests that MS withdrawal contributes to these alterations. Future studies will assess the effects of this treatment regimen on neurotransmitter levels in other brain nuclei, supported by grant DA0181-01, M. D. Schechter, P.I.


MDMA (5-20 mg/kg) has been shown to be neurotoxic to the serotonergic system with a minimal effect on the dopaminergic system. Subchronic treatment with low doses however, increased both serotonin and dopamine (DA) in specific rat brain nuclei (Maloney et al., Neurosci., Abst., 1987). To further investigate these findings, rats were administered either 1.5 mg/kg MDMA (B) or 1.0 ml/kg Hyo (W) i.p. for 6 weeks according to the following 2 week injection schedule: DVOVDDYO. Between 2 and 3 weeks after the last injection, DA release was measured in anterolateral caudate by in vivo microdialysis in these awake, behaving rats. Dialysis perfusion (2.5 μl/min) was with a modified modified Ringer's solution (pH 7.25). Samples were collected every 20 min and analyzed for DA, DOPAC, and HVA by HPLC/EC. After a hr stable baseline, all rats were injected with 1.5 mg/kg +MDMA. Dialysate samples were collected for 3 hrs. There was a significant increase in DA accompanied by a decrease in DOPAC. Forty min after MDMA, DA release in the subchronically treated MDMA group was significantly higher (204% of baseline + 19) than the vehicle-treated group (146% ± 6) (p<0.05). There were no differences in DOPAC levels between these groups at this time. These results may indicate a sensitization to the DA releasing properties of MDMA following an intermittent, low dose treatment regimen. Future studies will assess the effects of this treatment regimen on neurotransmitter levels in other brain nuclei. Supported by grant DA0181-01, M. D. Schechter, P.I.
ASSESSMENT OF SPATIAL VISION AND VISUAL FIELDS IN NATURALLY STRABISMIC MONKEYS. M. Quick, M. Josses, R.G. Boothe (SPON: J. Tiggges). Terkes Regional Primate Research Center, and Departments of Psychology and Ophthalmology, Emory University, Atlanta, GA 30322.

A screening program has yielded monkeys having a naturally occurring ocular misalignment. Its occurrence was often associated with a large tropic refractive error, and with a difference in refractive error between eyes. Quantitative estimate of the deviation was made from photographs. The results showed monkeys with 1) alternating fixation; 2) deviations that varied with fixation angle; 3) deviations that varied with fixation distance, indicating an accommodative component.

Spatial vision was assessed operantly. Grating acuity results showed a sensory deficit in the deviating eye of some monkeys with deviations up to one octave when compared to the fixing eye. Further deficits were seen in tests of optotype acuity. This further reduction correlated with deficits in phase discrimination and increased spatial distortion. Also, a loss of sensitivity to contrast was evident at mid and high spatial frequencies. The horizontal extent of the visual fields were determined using a perimeter apparatus. Deficits were noted within the monocular segment of some non-preferred eyes, indicating field losses in deviating eyes which are unaccounted for by typical competitive mechanisms. Correlations will be drawn between oculomotor state and behavioral results. NIH grants EY-06436 and RR-00165.


Human strabismic anophyles show an abnormal relationship between vernier acuity and grating acuity. We studied this relationship in strabismic monkeys to better understand the neural basis of amblyopia. In addition, we investigated the hypothesis that vernier acuity can be predicted from knowledge of the contrast sensitivity function.

We measured monocular vernier acuity, grating acuity and contrast sensitivity for two keys (Macaca nemestrina) made esotropic at ages ranging from 3 to 8 weeks. All data were collected using operant methods. The relationship between vernier acuity and grating acuity in the strabismic monkeys was different from that of normal adult monkeys. However, part of the abnormal response in the strabismic monkeys could be described by the performance of young normal animals. The deficits in vernier acuity were not obviously associated with the contrast sensitivity functions.


Amylloids are known to have a potency of lesions in primary visual cortex that can be visualized via an induced effect. We used PET (methodology: 18F binds to brain tissue) to investigate effects of visual stimulation on a glucose metabolism in cerebral cortex of normal and amylrophic human subjects under the tower (PET) frame. The visual stimulus was a monocularly-presented dramatic motion picture. Two control subjects had normal vision in each eye, while 3 amyllopies had monocularly decreased vision (30/200) and normal vision in the fellow eye. Rotational sensitivity was quantified by quantifying the area in terms of interest by activity total in the entire brain images.

Repeated scanning demonstrated similar relative glucose metabolism in primary visual cortex of a control viewing with either normal eye. Optical blur (~20/200) reduced relative glucose metabolism in primary visual cortex by ~8% in two controls. Four of 5 subjects exhibited asymmetry greater relative glucose metabolism in the right hemisphere.

These preliminary results indicate that amylloids reduce visual acuity of relative glucose metabolism in primary visual cortex of amylloids, but effect this may be stimulated by optical blur in normal subjects. In both normal and amylloids subjects, complex visual stimulation often produces asymmetry greater relative glucose metabolism in the contralateral hemisphere; this effect is reduced by optical blur.


Visually evoked potentials (VEP) were obtained from 45 healthy normal full-term infants from 4 weeks to one year of age, 6 normal and 6 amylrophic adults. The stimulus was pseudorandom binary sequence (PRBS), 0 to 66.7 Hz at 100% DB cut-offs ranging from 1 to 40 Hz. Both the Fourier amplitude spectra frequency maxima and F0 shifted towards higher frequencies with increasing age. However, adult levels were not reached by the end of the first year of life. These results may reflect the development of central nervous system mechanisms, whereas previous studies using fischer fusion may reflect retinal cone development. Differences between normal and amylrophic adults were found.
VISUAL CORTEX MAPS IN KITTENS WITH BILATERAL AMBLYOPIA. Max S. Cynader, Joanne A. Mandeville, Nicholas V. Souidian, Kenneth M. Morrison, and Donald E. Mitchell. Dept. of Ophthalmology, U.B.C. Vancouver, B.C. V5Z 3N9, and Dept. of Psychology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada.

We studied visual cortex functional topology in kittens reared under a condition which prevents vision. Kittens were reared with one eyelid sutured until five weeks of age. Thereafter, they had 9 or 18 days of reverse surgery, during which time the initially exposed eye was allowed vision. On p18, the initially exposed eye was occluded. Thereafter, both eyes were allowed normal vision. This results in a profound deterioration of vision in both eyes, with visual function being severely impaired by several factors. Our results show that the laminar organization of the visual cortex is preserved in the amblyopic visual system. These findings suggest that the visual cortex is able to adapt to changing visual conditions and may provide insight into the mechanisms underlying cortical plasticity.

A striking difference was found in receptive field size in the amblyopic, receptive fields averaged 60% larger than those of normal cats. This high significantly different, coupled with the equalities of point-spread function, leads to large differences in size of the area over which receptive fields overlap and thus in hypercolumns dimensions between normal and amblyopic animals.


Serum-free or defined media (DM) provide a more reproducible environment than serum-containing medium (SCM) for studying neural regeneration in vitro. We have devised several DM for primary neural culture and here report immunohistochecmical (IHC) and electrophysiological data. Spinal and dorsal root ganglion (DRG) cultures were prepared from 12-14 rat embryos and maintained at least 3 weeks. SCM was compared with several defined DM (proportional concentrations). IHC studies consisted of staining neurons (NSE, ascorcytine (GAP) and oligodendrocytes (GalC). The morphology and relative frequency of each cell type in the various media were noted. Electrophysiological recordings were made from DRG neurons. Resting potential, action potential amplitude, duration, and afterhyperpolarization were compared. At 4 weeks in culture, there were no significant differences between SCM and SCM cultures. Further comparisons of differing DM formulations are planned.
POSSIBLE NEUROTROPIC EFFECTS OF NIMODIPINE ON COGNITIVE NEUROMODULATION OF CATECHOLAMINERGIC GROWTH IN THE CHICK EMBRYONIC BRAIN BY TWO HYPOTHALAMIC NEUROPEPTIDES. S. Van derbilt Univ., Nashville, TN 37212

We have studied nerve fibers from patients with idiopathic muscle hyper trophy and this may be present in excess in patients with idiopathic muscle hypertrophy.

Supported by NIH-SCDA grant #NS01134 to Dr. Misulis and a VA research grant to Dr. Stochcheck.

500.5 MYOBLAST PROLIFERATION INDUCTED BY SERUM FROM PATIENTS WITH HYPOTHYROIDISM. R.K. Misulis, C.M. Stochcheck, & C.M. Stochcheck, Inst. of Molecular Biology and Medical Biotechnology, Univ. of Utah, Salt Lake City, UT 84112

Myoblasts incubated in serum from normal subjects had an increase in cell number over the 5 days to 574% of plated number. Patient serum resulted in an increase in myoblast proliferation to 1348% of the pre-incubation cell number. Serum levels of many hormones, including growth hormone, thyroxin, somatomedin C, were normal.

These data suggest that myoblast proliferation may be controlled by an as yet unidentified circulating factor, and this may be present in excess in patients with idiopathic muscle hypertrophy.

Supported by NIH-SCDA grant #NS01134 to Dr. Misulis and a VA research grant to Dr. Stochcheck.

500.7 NEUROMODULATION OF CATECHOLAMINERGIC GROWTH IN THE CHICK EMBRYONIC BRAIN BY TWO HYPOPTHALAMIC NEUROPEPTIDES. S. Kantrati and A. Vernadakis (SPON: D.W. Whitlock), Deps. of Pharmacology and Psychiatry, Univ. of Colorado Sch. of Medicine, Denver, CO 80262

We are investigating the neuromodulatory role of growth hormone-releasing factor (GRF) and gastrin-releasing peptide (GRP) on catecholaminergic neural expression in the chick embryonic brain. The activity of tyrosine hydroxylase, a limiting enzyme in catecholamine synthesis, was used as an index of neuronal development. The peptides were given either at embryonic days E1 to E7 or at E14 to E14, a period of synaptogenesis. In the first paradigm, animals received GRH (150µg) or saline vehicle (500µl) i.p. at E7 and E14 and assayed for TH activity on E15. Whole brains minus optic lobes were assayed for TH activity, expressed as pmol CO2 liberated/mg protein. The activity was significantly higher (p<0.01) in the treated embryos versus controls (6.8±2.7 vs. 4.8±3.58, respectively). We interpret these data to mean that GRH and GRP may be involved in catecholaminergic neural growth and synaptic function. (Support:NIH grant #AM07464)

500.8 TREATMENT OF GANGLIOSIDOSIS AND AMYLOIDOSIS FAIL TO REDUCE SPATIAL LEARNING DEFICITS IN A WATER-MAZE TASK FOLLOWING BILATERAL LESIONS OF THE CAUDATE NUCLEUS. G. L. Dunbar, L. Lescaudron*, B. S. Bitran*, S. A. Hecht*, and D. G. Stein, Brain Research Lab, Clark University, Worcester, MA 01610, Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Although gangliosides (GM1 and AGF2) and d-amphetamine reduce learning deficits following irreversible task after bilateral caudate nucleus (CN) lesions (Dunbar et al., Soc. Neurosci. Abstr., 12:1283, 1986), the effects do not generalize to a task which requires utilizing distal cues (i.e., the Morris water maze). Forty-one male, albino rats (Sprague-Dawley, 350-450g) were given sham operations and IP saline injections (n=9) or bilateral CN lesions, followed immediately by IP injections of either 20 mg/kg GM1 (n=9), 20 mg/kg AGF2 (n+7), or 2 mg/kg d-amphetamine (n=7) or IP saline (n=9). Injections were given daily for 10 days. Five days after surgery, testing began in the Morris water maze and in an open field twice a day, for five days. Some of the treatments were able to reduce learning deficits on the water maze task. Also, no between-group differences were found in open-field activity levels. These results suggest that the beneficial effects of gangliosides and amphetamines may include task-specific in reducing spatial learning deficits following CN lesions.
Trophic support of 5-HTR neurons in vitro

D. P. Eysse and J. Patrick
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Pure cultures of central neurons in which all cells were stained positive for nerve filaments were obtained from dissociated chick forebrain on embryonic day 8. The cultures were grown for 1, 5 and 7 days in vitro either on untreated tissue culture plastic or on poly-L-lysine precoated plates. We observed trophic support of bovine serum albumin (BSA) and ovalbumin (OA) when the serum free medium in the culture plates was changed from 50 μM to 1.5 μM and from 0.87μM to 28 μM, respectively. At the plateau level of BSA (75μM) 66% of the cells survived for more than 24 h of incubation, whereas the plateau level of OA (13.5 μM) only 52% of the cells survived. This neurotrophic support exceeded by far our observation of the effects of pyruvate/catalase in the presence of a buffer in the short term cultures. Only the albumins provided an extended neurotrophic support (7 days) which was accompanied by the appropriate neuronal sprouting.

Neuronal survival was estimated by means of a strip-counting technique and an automated colorimetric microassay (Manthorpe, N., et al., Develop. Brain. Res. 25: 191, 1986). Albumins of a greater purity have excluded the possibility of essential fatty acids being responsible for the described effects. It remains to be determined if the forms of somatotropins: IGF-I and IGF-II (Carlsson-Skwirut, C., et al., FEBS 201:46, 1986) are the trophic substances that permit not only the longevity of the neurons, but also the normal maintenance in culture.

5,7-DHT-lesion-induced neurotrophic factors increase survival in vitro. D. C. Tanzi

A recent study (Tanzer, R., et al.; Kitaguchi N. et al.; Nature. 331:525-532, 1988). It showed that thrombin, but not other proteases tested, promotes neurite formation in normal cerebellar cultures. Finally, highly concentrated NT-3 may contain a factor inhibiting neuronal growth. Supported by NIH NS24524 and DE07734.

The present study showed that PN-1 activity was eight-fold lower in autopsy brain samples from 10 Alzheimer’s disease cases compared to 6 control cases. PN-1 activity was quantitated based on its ability to form SSS-resistant complexes with 125I-thrombin that were blocked by a specific anti-PN-1 monoclonal antibody (Wagner, S.L., Van Nostrand, W.E., Lau, A. and Cunningham, D., Biochemistry. 27:2173, 1988). The much reduced PN-1 activity in Alzheimer’s disease samples was not due to differences in postmortem delay, age or sex. We suggest that the reduced levels of PN-1 in Alzheimer’s disease brain could result in increased thrombin levels that in turn could lead to disrupted interactions among neurites and altered neurite morphologies.


The biochemical basis for the neuroprotective effects and the loss of cognitive functions that characterize Alzheimer’s disease are unknown. The recent findings that brain amyloid deposits of Alzheimer’s disease contain trypan and chymoquinins inhibitors indicate that an imbalance of proteases and protease inhibitors may be involved (Ponti, P. et al.; Tanzi, R., et al.; Kitaguchi N. et al.; Nature. 331:525-532, 1988). It was recently shown that thrombin, but not other proteases tested, promotes neurite formation in normal cerebellar cultures. Finally, highly concentrated NT-3 may contain a factor inhibiting neuronal growth. Supported by NIH NS24524 and DE07734.

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ACTION OF GANGLIOSIDE ON RESPONSIVENESS OF SENSORY GANGLIA TO TROPIC AGENTS. D. P. Eysse and J. Patrick

Department of Anatomy and Neurobiology, School of Med., Univ. of Louisville, Louisville, KY 40292.

Gangliosides potentiate the action of Nerve Growth Factor (NGF) on chick embryonic 9 day (ED) sensory ganglia (DIG). The responsiveness of DIG neurons to NGF begins on ED 7, is maximum on 9 and declines totally by ED 13. To determine if other bovine serum albumins (BSA) and ovalbumin (OA) would also potentiate NGF during developmentally reduced responsiveness, DIG (ED 6-14) were explanted in Medium 199 containing 10% heat-inactivated fetal bovine serum. The DIG neurons contained a small amount of G-1 (150 μg/ml) in the presence or absence of BSA (1 μg/ml). Neuritogenesis was evaluated in terms of neurite number and the amount of the protein product. No G-1 potentiation was observed during the peak of NGF-mediated development (ED 8-10) on other days, treatment with DIG (ED 9) in medium conditioned by unstimulated C6 glioma cells for 3 days (GCM) stimulates DIG in the presence of anti-NGF, the ability of GCM to potentiate G-1-mediated growth was examined (ED 6-14). Maximal potentiation of GCM by G-1 occurred on ED 9-10 and the concentration of NGF was submaximal. GCM enhanced the trophic support when its activity was submaximal by altering the extent but not the duration of the response. The studies demonstrate that gangliosides depend on a regulatory role in trophic interaction. Supported by NIH NS24524 and DE07734.


We report here for the first time that variations in the levels and timing of insulin exposure immediately after birth can differentially affect the morphological patterns of astroglial differentiation as well as expression of GFAP in long-term organotypic cultures of E-17 mouse cerebellum. The medium was supplemented with no insulin, low insulin (10 pg/ml) or high insulin (10 μg/ml). Cultures were stained immunocytochemically with monoclonal antibodies to GFAP or prehybridized in the presence of biotinylated cDNA GFAP probe. High insulin elicited an increase in GFAP mRNA and intense GFAP immunoreactivity. Low insulin produced no increase in GFAP mRNA or message. The very morphology of GFAP+ cells was also influenced by the hormone concentration in an age-specific manner. Facial radial glia were expanded and comprising 60% of GFAP+ cells in contrast to a reversal of this pattern by low insulin, where 60% of GFAP+ cells were flat cells. In newborn cultures, the basement membrane-like structures to high and high insulin were considerably attenuated. In view of the critical dependence of interactions of developing neurons with radial glia for neuronal migration, differentiation, and the initiation of neurite growth, these changes in morphology suggest developmentally-regulated mechanisms by which insulin-related peptides may influence directly and indirectly neuronal and astroglial differentiation.
Gangliosides can regulate the neuritogenic and neurotrophic development of several types of embryonic neurons and neuroblastoma in vitro. We have shown that individual gangliosides produce subtle differences in the neuritic patterns. To determine the minimum molecular structure required to produce these changes, we examined the effect of exogenous phosphatidic acid (PA) on neuritogenesis. PA was suspended in phosphate buffered saline, sonicated, diluted with nutrient medium at various concentrations (3.3 x 10^-3 to 3.3 x 10^-4 M) and applied to Neuro-2a cells. Growth was evaluated in terms of neuritic complexity and cell processes. PA was stimulatory over a broad concentration (maximal at 3.0 μM) at 24n. An index of PA's metabolic action was obtained from ornithine decarboxylase induction. Maximal activity was obtained at 30 μM. To determine if PA could potentiate Herve Growth Factor (NGF)-mediated neuritogenesis, the effect of PA on NGF-mediated development of rat pheochromocytoma PC-12 cells and chick embryonic dorsal root ganglia (DRG) was examined. PA had a moderate effect on PC-12 and DRG differentiation. These data demonstrate that although the responses were not equivalent both PA and GM1 enhance neuritogenesis. Supported by NIH NS24524 and DE07734.

A RAT HYPOTHALAMIC CELL LINE WHICH IS SENSITIVE TO SEVERAL DIFFERENT TISSUE GROWTH FACTORS. J. Torres-Aleman*1, F. S. Klett*2, and S. J. Kohlbiel*2. (SPON: F. Spencer). Sect. of Neuroendocrinology, Dept. of OB/GYN and Medicine, Yale Medical School, New Haven, CT 06510.

Trophic factors affecting brain cell development appear to be cell-type specific. A single neuron may respond to different growth factors as a function of its developmental state. We examined the possibility that hypothalamic cells may possess responsiveness to more than one growth factor. A stable PC12 transformed rat embryo hypothalamic cell line (F-12) was grown in the presence of either FGFb, insulin, IGF-I or MSA (from 0.1 pM to 10 nM). Dose dependent growth promoting effects were present in the following potency: FGFb, IGF-I = insulin, MSA had no effect. At 0.1 nM FGFb, produced a 2 fold increase in cell number, IGF-I a 10 fold, and insulin a 9 fold. ED50 was between 0.1 and 1 nM for all three peptides. We suggest that development of hypothalamic cells may occur in part via regulated or pre-programmed expression of a repertoire of growth factor receptors.

MAGNOISIDES APO2 PROMOTES RECOVERY OF AF64A-INDUCED BEHAVIORAL AND NEUROCHEMICAL DEFICITS. P.P. Emerich, M.J. Spates1, and T.J. Walsh. Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

AF64A is the internal ester of GM1 and has been shown to promote recovery of function following central nervous system injury. The studies presented here examined the effects of APO2 on the behavioral and neurochemical alterations induced by intraventricular administration of AF64A. This cholinesterase produces a marked increase in the activity of choline acetyltransferase (Chat) in the hippocampus (HPC) together with persistent cognitive impairment.

Sprague-Dawley rats were housed on a standard eight arm radial maze (RAM) task. Following training, rats were injected (IP) with 10 mg/kg APO2 or 0.9% saline for 3 days prior to and for 14 days following the unilateral injection of AF64A (500 μg/injection) or artificial CSF. Rats injected with AF64A (AF64A/CSF) were markedly impaired in their performance of the RAM task. In contrast, animals receiving APO2 (AF64A/APO2) were initially impaired but rapidly regained the task and performed as well as controls.

Rats were then trained to perform a working memory version of the RAM task in which they had to remember which 4 of the 8 maze arms they obtained food from prior to a one hour delay. Following the delay the rats were returned to the maze and allowed to choose freely among all 8 arms. Arms not previously chosen were baited, and entry into previously entered arms constituted an error (delayed-non match-to-sample). APO2-treated rats, whether treated with APO2 or not were profoundly impaired on this version of the task and showed no evidence of recovery. AF64A produced a 35% decrease in hippocampal Chat activity (AF64A/CSF) that was significantly ameliorated by prior treatment with APO2 (23% decrease). These data suggest that, in this model system, APO2 promotes recovery of function by either limiting the initial effects of AF64A or by facilitating some reorganization following the insult.

Supported by BRSG Grant (PHS 07058-11) to T.J.W.
Enhanced expression of the dynorphin gene has been observed in several CNS regions during specific types of stimuli. For example, activation of the rat dynorphin gene for regions that bind such regulatory proteins using a unique binding/exonuclease digestion procedure (Quinn et al Mol Cell Biol, 7:2735, 1987). A 3' end labeled 2.400 base fragment of the rat dynorphin gene plus the θC operator was then digested with a restriction enzyme that recognizes a specific sequence in the dynorphin gene. The θC fragment was separated from the remainder of the rat dynorphin gene by its unique sequence in the θC operator.

Comparison with peripheral tissues and several cultured cell lines showed one of the sites to be neural specific. Binding of all proteins could be competed by excess unlabeled dynorphin gene but not by poly-dAdT or poly-dIdC or several oligonucleotides. The method provides a rapid procedure to scan large gene fragments for DNA binding proteins which may participate in transcriptional regulation. These results demonstrate the presence of a neural specific protein that recognizes a specific sequence in the dynorphin gene.

Previously, we have characterized the tyrosine hydroxylase (TH) gene in several species, but the expression of this gene is not restricted to DA neuroblastoma cells. TH is a highly conserved enzyme that is responsible for the synthesis of dopamine from tyrosine. It is expressed in the hypothalamus, pituitary, adrenal medulla, and sympathetic ganglia.

Characterization of the rat thyrotropin releasing hormone (TRH) gene in several species, including human, mouse, and rat, has been reported. The TRH gene is highly conserved across species, and its expression is ubiquitous in the brain, with high levels in the hypothalamus and pituitary gland.

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501.7


Neuronal stimuli modulate the expression of phenylethanolamine N-methyltransferase (PNMT) in vivo. In vitro, we found that bovine adrenal chromaffin cell levels of PNMT mRNA increase (5-8 fold) in response to K+ depolarization (Neurosci. Lett. 13:1096, 1987). We sought to identify a region of the PNMT promoter which mediates the response to K+. PNMT 5' flank (1.1 Kb) including the transcriptional start (CAP) site was isolated from a rat genomic library (G. Scherr), probed with rat PNMT cDNA. This fragment was sequenced and the CAP site was determined by primer extension analysis to be 22 bp downstream of the TATA box. PNMT fusion gene constructs with a chloramphenicol acetyltransferase (CAT) reporter were made using pBlCAt2 or pBlCAt3 (B. Luckow). Plasmid pBl900 contains the entire PNMT fragment in the promoterless pBlCAt3 while pBl900 contains the most distal 480 bp ligated onto the minimal thymidine kinase promoter of pBlCAt2. pBl900 was introduced into rat C6 glioma cells with the neomycin resistance marker phage and stable transformants were selected in G418. Several stable lines consistently displayed a 2-4 fold increase in CAT activity when treated for 16 hrs with 50 mM K+, compared to matched Na+ controls. pBl900 was co-transfected with pRSVßgal into C6 cells and transients were treated with K+ or Na+ depolarization for 16 hrs with 50 mM K+, compared to matched Na+ controls. pBl900 contains the entire PNMT fragment in the promoterless pBlCAt3 while pBl900 contains the most distal 480 bp ligated onto the minimal thymidine kinase promoter of pBlCAt2. pBl900 was introduced into rat C6 glioma cells with the neomycin resistance marker phage and stable transformants were selected in G418. Several stable lines consistently displayed a 2-4 fold increase in CAT activity when treated for 16 hrs with 50 mM K+, compared to matched Na+ controls. pBl900 was co-transfected with pRSVßgal into C6 cells and transients were treated with K+ or Na+. CAT values were normalized to β-galactosidase activity. The pBl900 constructs increased CAT expression 2.4 fold in response to K+. This suggests that an element responsive to depolarization is present in the region between 400-900 bp, relative to the CAP site, of the PNMT promoter.

501.8


ARPP-16 (a phosphoprotein substrate for cAMP-dependent protein kinase, with an Mr of 19,000 (ARPP-19), was found to cross-react with the antibodies prepared against ARPP-16. Immunohistochemical analysis indicated that ARPP-16 was enriched in the basal ganglia while ARPP-19 was present in similar levels in all brain regions studied and was also present in non-neuronal tissues. cDNA clones were isolated from a bovine caudate cDNA library using a 31 bp oligonucleotide probe. An internal 16 bp sequence of the cDNA hybridized with oligonucleotide probes designed on the basis of the amino acid sequences of several peptides purified from chromotrophic digests of ARPP-16. Two distinct cDNA clones were isolated and the nucleotide sequences determined. Comparison of the nucleotide sequences and amino acid sequences indicates that one clone codes for ARPP-19 while the other codes for ARPP-16. The amino acid sequences of ARPP-16 and ARPP-19 are identical except that ARPP-19 has an additional 16 amino acids at the N-terminus. The two cDNA clones share an identical 3 untranslated region of 756 nucleotides. In addition, the cDNA clone for ARPP-16 contains 806 nucleotides following the common sequence. The 5' untranslated regions of the two clones are entirely different. These results suggest that ARPP-16 and ARPP-19 may be produced by tissue- and brain region-specific, alternative splicing of a primary transcript.

Supported by USPHS Grant MH 40899.

501.9

ISOLATION AND CHARACTERIZATION OF GENES SPECIFICALLY EXPRESSED IN THE ELECTROMOTOR NUCLEUS OF TORPEDO. M. Linial*, J. Meerow, and R.H. Scheller. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Polynucleotides antibodies raised against purified cholineriic synaptic vesicles from Torpedo californica were used to screen a cyt-11 expression cDNA library constructed from the electromotor nucleus (Carlson, S.S., and Kelly, R.B. (1980) J. Cell. Biol. 97, 98-103). Three of the cDNA clones were shown to be specifically expressed in the electromotor nucleus and not in the electric organ, gill, muscle, heart, skin or liver. Moreover, the level of expression is approximately 10 fold higher in the electric lobe relative to the brain. The cDNA clones hybridize to three independent mRNAs of 10 kb (clone 2), 5.5 kb (clone 3) and 2.0 kb (clone 4). Searches of the protein data bank using amino acid sequences predicted from the nucleotide sequences of the cDNAs did not reveal any significant homologies. Antibodies raised against the coding region of clone 2 recognize a unique protein with an apparent molecular weight of 350,000 daltons which copurifies with Torpedo synaptic vesicles.

502.1

CORTICAL DYNAMICS OF FORM AND COLOR PERCEPTION. S. Grossberg* and E. Mingolla*. Center for Adaptive Systems, Boston Univ., Boston, MA 02215.

A model cortical architecture is described for explaining key processes of boundary detection, sharpening, regularization, and completion; shape-from-texture; and filling-in of brightness and color. The ar­

502.2

MAXIMUM INFORMATION PRESERVATION: A PROPOSED ORGANIZING PRINCIPLE FOR CERTAIN ASPECTS OF PERCEPTUAL NEURAL ARCHITECTURE. R. Linsker. IBM T. J. Watson Research Center, Yorktown Heights, NY 10598.

What principles might account for the strikingly complex sets of feature-analyzing properties found in mammalian perceptual systems, and for their organization and integration? A Hebb-type modification rule causes model cells in a feedforward network to develop so that (under certain conditions) each cell's output activity conveys maximum information about its input activity values [R. Linsker, Computer 21(5): 105 (March 1988); see also Proc. Natl. Acad. Sci. USA 83: 7506, 8390, 8779 (1986)].

This suggests a potential organizing principle, 'maximum information preservation,' for many high-level perceptual processes. It might be that a Hebb-type rule can be a part and/or other developmental mechanisms.

For certain simple ensembles of input activities, this principle generates topographic maps, 'cortical receptive fields' which are an aspect of the feedforward network developing into a layered neural network having feedforward and lateral (intralayer) connections. According to this principle, each processing stage develops so that the output signal values (from that stage) jointly convey maximum information about the input values (to that stage), subject to certain constraints. (The quantity that is maximized is a Shannon information rate.) This principle may be implemented by activity-dependent changes in which a Hebb-type rule can be a part and/or other developmental mechanisms.

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SUDDEN COLOR-BLINDNESS OF CEREBRAL ORIGIN. O. Sacks*. R.L. Was-
associated, at autopsy, with vascular or other lesions of visual cortex. Such lesions have
have visual field defects, visual alexia and some degree of form agnosia (Pearlman et
also been demonstrated in life by neuro-imaging. The majority of such patients also
determination of pattern motion may occur there [1]. By examining how the
temporal area of extrastriate visual cortex suggesting that the neural
which respond selectively to pattern motion have been found in the middle
imagery and recall, now found that his recalled images were equally void of color. With
by the motion of the two grating components within a plaid (the constraints rule)
substantially and systematically with the contrast ratio of the grating components.
HUMAN PERCEPTION OF 3-D STRUCTURE FROM MOTION:
mental-agnosia, and no defects in the visual fields (at least when seen by us ten weeks
after his injury). More formal testing with color 'Mondrians' (Land, 1977) suggested
estimation speed of the components is subject to a contrast-dependent distortion
for both 2- and 3-D stimuli, perceived velocity was greater with shorter
lifetimes and velocities tested. These results suggest that the computation of 2-
drifting sinewave gratings were used with a method of constant stimuli to obtain velocity
discrimination thresholds as a function of stimulus duration for a range of velocities and spa-
tial frequencies. The predicted results on the basis of the above physiology would be that this
function should be non-monotonic, exhibiting a local minimum corresponding to an optimum dis-
place-ment. For stimuli of different spatial fre-
quencies, we determined that a Dopt rule could emerge which can be expressed in terms of a certain fraction of a
spatial cycle. Our results show that there are indeed local minima in the velocity discrimination function and that the Dopt rule that emerges corresponds to 1/6 of a spatial cycle or integral multiples of
this. This is in agreement with the single cell results cited above.

Zihl et al (1983) have described a "motion-blind" patient, who had suffered a specific loss of visual motion perception resulting from a vascular failure. Other visual functions, such as scotoly, color vision, critical
ticker fusion frequency, and stereopsis, were normal. We have used discrimination psychophysical methods and sinewave grating stimuli to further characterize this
patient's deficit.

Contrast sensitivity to detect the presence of a
grating, as a function of its spatial and temporal frequency, was found to be only slightly impaired. However contrast sensitivity to discriminate the direction of a motion grating showed severe reduction.

For suprathreshold gratings, contrast discrimination and spatial frequency discrimination were only slightly affected, even if the gratings were moving. However, temporal frequency or velocity discrimination of these
gratings was severely impaired.

These findings are consistent with an extrastriate locus of damage.
Supported by Max-Planck-Institute Welcome Research Grant to R.H., and Canadian NSERC Grant to C.B.
502.9 CONTRIBUTION OF DIFFERENT PATHS OF THE CAT VISUAL SYSTEM TO DETECTING CORNER FEATURES. R. L. FIELD, J. COHEN, A. G. ARENS, AND H. ROB, University of California, San Diego, La Jolla, CA. 

The contributions of inputs from the retina and from other sensory and motor systems to the detection of corner features in the visual field were investigated. The results suggest that the contributions of these inputs are not independent and that the detection of corner features involves a complex interaction of these inputs.

502.10 VISUAL REACTION TIME OF THE CAT AS A FUNCTION OF SPATIAL FREQUENCY. M. L. TUTTLE, W. W. HALL, AND K. E. ANDES. Dept. of Psychology, University of Maine, Orono, ME.

The visual reaction time of the cat was measured as a function of spatial frequency. The results indicate that the cat's visual reaction time is not simply a function of the spatial frequency of the stimulus, but is also influenced by the spatial frequency of the background.

502.11 TEXTURE DISCRIMINATION AND PERCEPTION OF COGNITIVE CONTOURS IN CATS BEFORE AND AFTER CORPUS CALLOSUM LESIONS. J. J. ROBBINS, H. KUDEL, AND J. KAUFMAN. Albert Einstein College of Medicine, Bronx, NY.

Texture discrimination and perception of cognitive contours were studied in cats before and after corpus callosum lesions. The results indicate that the corpus callosum plays a significant role in the processing of texture and contour information.


The temporal dynamics of binocular rivalry were studied using a psychophysical model. The results suggest that binocular rivalry is a reciprocal inhibition process.


The analysis of localized and distributed processes using one-dimensional current source density techniques was studied. The results indicate that convolutional and sampling considerations are important in the analysis of these processes.

502.14 SEQUENCE AND DISTRIBUTION OF PATTERN-EVOKED ACTIVITY IN AREA 17 OF THE HABITUATING MONKEY. C. E. SCHNEIDER, M. E. FERGUSON, AND H. DAVISON, Deps. of Neurosurgery and Neurology, Albert Einstein College of Medicine, Bronx, NY.

The sequence and distribution of pattern-evoked activity in area 17 of the habituating monkey were studied. The results indicate that the activity is not randomly distributed, but follows a specific sequence.

502.15 VISUAL CORTEX VII

Steady state Evoked Potentials (EPs) in response to sinusoidally modulated light can be described and analyzed in terms of frequency selective processes. These frequency selective processes have amplitude peaks in the low, medium, high frequency region at about 10, 20 and 40 Hz (Spekreijse et al. Visual Evoked Potentials in man: new developments, J.E.Dexerstedt ed, Oxford University Press, 1977).

This study deals with the topographical distribution and the location of the neuronal generators of luminance EPs in man, of which the harmonics are plotted on the topographical maps. The analysis is based on the assumption that in each distinct frequency-region the potential distribution of the underlying cortical activity can be ascribed to a single current dipole. A precondition for computing an equivalent dipole from topographic maps is that the phase of the harmonics in all derivations is the same; only phase changes of 180 degrees are allowed.

In correspondence with earlier findings we could confirm that the source of the evoked activity in the high frequency region is located in the primary visual cortex and that this activity can be described by an equivalent dipole. Further, we found that the recorded potential distribution in the medium-frequency region also can be ascribed to a single current dipole. This equivalent dipole shows a similar behaviour as the one representing the contrast sensitive response. The orientation of the dipole is radial for visual stimulation and changes tangentially with increasing stimulus eccentricity. The activity evoked in the low frequency-region is not specific and the source seems to be situated deep in the head.

502.16 AN ANISOTROPIC MULTI-SPHERE MODEL FOR SOURCE LOCALIZATION BY VEPs IN THE SEPTUM, C. de Menocal, B. Scott, and H. Spekreijse. (SPON: P.A. Apkarian). The Netherlands Ophthalmic Research Institute, F.D.Box 12141, 1100 AC Amsterdam-ZO, The Netherlands.

When multi-channel visually evoked potentials (VEPs) recordings are used for the localization of brain activity mathematical models are needed that describe the electrical properties of the head. Generally it is described as a volume conductor with a point source and a sphere. None of the present models take into account the anisotropy of the various parts of the head, although anisotropic conduction has been demonstrated in various parts of the human head. The anisotropy ratio changes from a factor of ten for the skull and the white matter, and more than a factor of two for cortical tissue. Further, the present analytic solutions of volume conductor model are restricted to a limited number of shells (four) and a spherical geometry. It is clear that the use of a simple model may lead to systematic errors in the source localization procedure. Therefore a more general solution has been found, which includes the effects of anisotropy, the arbitrary number of shells and the fact that the head may better be described by a spheroid than a sphere. The solution was obtained by generalizing the method of Morse and Feshbach to obtain a Green's function of the Laplace equation. The potential distribution due to a dipole source is then found by taking the gradient of the Greens function and determining the inner product of the result and the dipole vector. It is possible to present the formulas in a convenient form, such that if they are applied in practice the likelihood of software errors is minimal.

502.17 VEP EVIDENCE OF FUNCTIONAL DIFFERENCES IN UPPER AND LOWER VISUAL FIELDS IN MAN. Fred H. Pervel. USAF School of Aerospace Medicine, Brooks AFB, TX.

This study investigated differences in the functional characteristics of two components of the pattern-reversal visual evoked potential (VEP) in humans: N1 (which is elicited primarily by upper visual field stimulation) and P1 (which is generated by lower visual field stimulation). The stimulus parameters which were used to isolate the two components were grating spatial frequency (1, 4, 8, 32 cpd) and contrast (0, 15, 40%). VEPs were recorded monopolarly from eight subjects using O2 as the source electrode site. Square-wave gratings, counterphase, were presented at one of two rates equal to or faster than each VEP. The gratings were presented at a mean luminance of 15 cd/m² and were contained in full-field as well as hemifield presentations. The VEP data revealed the existence of bandwidth spatial tuning and little contrast saturation in the case of N1 and lowpass spatial tuning and a non-linear contrast characteristic in the case of P1. In general, N1 may be viewed as largely reflecting parvocellular processing in the visual cortex, whereas P1 more finely reflects extraretinal output. Thus, these VEP findings parallel recent anatomical and physiological studies (Burkhalter, Felloman, Heussen, and Van Essen, Vision Research, 1980, 20, 63-80) in the processing of information in the upper and lower visual fields.

502.18 PATTERN VEWOKED POTENTIALS FROM CHILDREN UNDER GENERAL ANESTHESIA. E.S. Fox, K. W. W. White, and K.J. Eriksen*. University of Southern California School of Medicine and Children's Hospital of Los Angeles, Dept. of Ophthalmology, Los Angeles, CA, 90034.

Pattern visual evoked potentials (PVEPs) were recorded from 9 normal eye subjects, 9-10yrs of age, under general anesthesia. Children were anesthetized with either trachéal intubation, with Midazolam (0.6%/Framar 0.3%) or midazolam and nitrous oxide (100%/Framar 0.3%) or Succinylcholine (0.6%). VEPs were recorded from 12 scalp electrodes from 3 recording positions: Fpz, C3 and C4. The eye was kept open by the experimenter, vision was corrected and fixation was monitored continuous. Alternating (2Hz) black-white checkerboard stimuli with check sizes ranging from 32-54 arc minutes of arc were used. Transient evoked responses were digitized and averaged. A minimum of 100 trials were obtained for each check size. In two subjects we were unable to obtain PVEPs due to the presence of alpha waves. Results from the remaining 7 subjects revealed that the mean P100 latency increased from 121ms with 55ms checks to 186ms with 14ma checks. Mean P100 amplitudes decreased from 38±13 to 27±6µV over the same stimulus range. The mean visual threshold obtained was 8.75ma. In general, the waveform obtained had a broad peak. Four children sedated with chloral hydrate showed PVEPs with similar latencies but smaller amplitudes than were obtained under anesthesia. The results suggest that PVEPs can be reliably obtained to small spatial frequency stimuli under anesthesia, and may be important in the clinical visual assessment of children.


Species comparisons can indicate the extent to which visual system functions are similarly simple and demonstrate the adequacy of experimental animal models. We measured steady-state visual evoked potentials in rats and humans in response to gratings of different spatial and temporal parameters. Spatial frequency of sine-wave gratings spanned 0.05-0.8 cycles/degree (cpd) for rats and 0.5-8.0 cpd for humans; temporal frequency of on/off square-wave modulation spanned 3-20 Hz; and contrast was 20%. Spatial amplitude of the averaged responses was measured at one (IF) and two times (2F) the stimulus rate.

The maximum IF amplitudes usually occurred at intermediate spatial frequencies approximating the respective contrast sensitivity function peaks of both rat and human. The 2F amplitude was usually maximal at low spatial frequency. At frequencies greater than about 20 Hz, both species exhibited a drop in 2F and an increase in IF amplitude, apparently reflecting a response transition from 2F to IF.

In summary, (1) rat and human visual systems responded in a qualitatively similar fashion to spatial and stimulus manipulations, and (2) IF and 2F showed different spatial and temporal profiles suggesting contributions of functionally distinct generators. (RKS supported by National Research Council Research Associateship).


A prominent feature of flash evoked potentials (FEPS) recorded from rat visual cortex is a late negative wave with a peak latency of about 155 msec (N155). Depression of N155 amplitude occurs following treatment with a variety of toxic and pharmacological agents. This depression shows a large variation upon the relative importance of changes in behavioral state (habituation to the test environment) and stimulus parameters (flash rate and contrast). These effects are generally described as determinants of N155 amplitude. All studies were performed upon unanesthetized adult male Long-Evans hooded rats with previously implanted electrodes. Visual cortex (VC). Four flash rates (0.5, 1.0, 2.0 and 4.0Hz) and two background illumination levels (0 and 115 lux) were used to study habituation. Flash rate was investigated by varying the time in the test chamber before testing (1, 2, 4, and 8 min) and by repeated testing (8 test sessions over 3 days). Amplitude were measured from baseline. The most critical determinant of N155 amplitude was number of test sessions. As number of trial sessions increased, so did N155 amplitude, which approached asymptote by the final session. Flash rate did not influence, and testing in the dark slightly reduced N155 amplitude. We conclude that although the N155 peak is elicited by a sensory stimulus, its amplitude depends more upon the behavioral state of the animal (i.e. habituation to the testing) than upon stimulus parameters.

We used cresyl violet, hematoxylin-eosin/Luxol fast blue, silver impregnation and immunostaining of the glial marker--GFAP--to examine the selectivity of neuronal death in gerbil neocortex after varying both the duration of bilateral common carotid occlusions and the post-ischemic survival time. A specific laminar pattern was consistently found in the post-ischemic somatosensory (3M) cortex. Six hours after a 5 minute occlusion, fibers and a few small- to medium-sized degenerating pyramidal cells were found in lower layer III and upper layer VI. After the following two days, neuronal death in these layers spread from the facial vibrissa--representation area to other areas within 3M cortex. This basic laminar pattern persisted up to five weeks (the longest time studied). Occasionally, degenerating cells and fibers were found in the auditory cortex but not in the motor or visual cortex. The pattern of GFAP reactivity also correlates with this laminar selectivity. Finally, degenerating cells were found in all layers of cortex including the large pyramidal cells in layer V after 15 minutes occlusions. Supported by NIH NS06533.


We used a Morris water-maze to determine if gerbils used spatial memory to solve the maze and if gerbils developed a defect in spatial memory after transient forebrain ischemia. Animals were given a hidden platform that was either stationary (PLACE) or moved with each trial (RANDOM). Four trials were given each day and the latencies in seconds for the animal to find the platform were recorded. Latencies for both groups decreased with repeated trials (p<0.001). After the 12th trial, the PLACE animals had significantly lower latencies than the RANDOM animals (p<0.03). PLACE animals repeatedly crossed the spot where the platform had been (p<0.01) when the platform was removed, while RANDOM animals did not swim in any preferred quadrant (p=0.47). To study the effect of ischemia, gerbil were grouped: ISCHEMIC (5 min carotid occlusion, testing 7 days later), CONTROL (sham surgery or no surgery), and RANDOM (no surgery, random platform placement). All groups showed decreased latencies with repeated trials. The RANDOM group had longer latencies in the last four trials then the two groups with fixed platform placement (p<0.05). There was no difference between the final latencies of the ISCHEMIC and CONTROL groups. These data show that: 1) gerbils are capable of solving the Morris water-maze, 2) gerbils use spatial memory to escape to a hidden platform and 3) CA1 damage in the gerbil does not disrupt this performance. These are two possible explanations for our findings: CA1 may not be necessary to solve the maze, or the hippocampus may not subserve spatial memory in the gerbil. We prefer the hypothesis that CA1 is not required for solving the maze. (Supported by the V.A. and NIH, NS06233).

503.3 CHANGES IN SOMATOSENSORY EVOKED POTENTIALS DURING GLOBAL BRAIN ISCHEMIA IN THE RAT. E.A. Pinkhasov*, A.Y. Frinkers and R.R. Baray (SPFN: R. Howland). Section of Neurosurgery, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

Somatosensory evoked potentials (SEPs), mean blood pressure (MBP) and heart rate (HR) responses were recorded in male Wistar rats anesthetized with urethane (1g/kg, iv) and artificially ventilated. Global brain ischemia was induced by the bilateral ligation of vertebral arteries at C1 level followed by bilateral occlusion of common carotid arteries for 3, 6 and 12 min. Control BP and HR were 99.6 ± 12.6 mmHg and 423.1 ± 60.7 bpm, respectively. Following control SEPs were obtained at 3, 6, and 12 min post-ischemia with 3 major peaks (P1, P2 and P3) with the onset of 6.8 ± 0.6, 11.0 ± 1.4 and 16.9 ± 1.4 msec, respectively. The amplitudes of these peaks were 0.2 ± 0.1, 2.3 ± 1.7 and 2.0 ± 1.4 uV mV. Global brain ischemia for 3, 6 and 12 min, produced an increase in MBP (42.5, 60.0, and 42.5 mmHg, respectively). Concomitantly there was a decrease in the amplitude of first two peaks (P1 and P2) while P3 disappeared. The changes in BP and SEPs in response to global brain ischemia for 3 min, but not for 6 and 12 min, were reversible. This preparation may serve as a model for global brain ischemia. Supported by NIH (HL24347) and AHA(NJ).


Flunarizine, a frequently used antihistaminic drug, with topical illumination of the skull causes photo-oxidative injury to cerebral vessels, and focal cerebral necrosis (Watson, B.D., Districh, W.D., et al., Ann. Neurol., 17:497, 1985; Van Reepsts, J., et al., Stroke, 18:1111, 1987). Does this model of cerebral thrombotic stroke produce enduring neurologic deficits? And, if so, can these be mitigated by the calcium IV calcium antagonist flunarizine? We made unilateral infarcts in the hindlimb area of the parietal cortex. Flunarizine (1.25 mg/kg iv or 40 mg/kg po) or its solvent was administered 30 min after infarction. We measured neglect, limb placing reactions, and limb usage on elevated beams. On day 21, all rats were scored using a validated test to determine visual and thalamic glial reactivity (typically in ipsilateral nl. VPL and posterior). All control rats displayed sustained deficits in tactile and proprioceptive abilities as well as in the contralateral hindlimb. By contrast, placing deficits were dramatically curtailed by flunarizine (FL): 70-75 % of FL rats placed contralaterally on day 1, while the others recovered within 3 to 5 days; but 21-day old infract volumes and total thalamic density were unaffected. Following cortical infarcts, flunarizine preserves behavioral function within a critical period.

503.5 PREVENTION OF MEMORY DEFICIT AFTER ISCHEMIA BY POST-ISCHEMIC INSULIN. C.L. Voll, L.Q. Whisah and B.N. Amer. Neuroscience Research Group, University of Calgary, Calgary, Alberta, T2N 4N1, and Dept. of Psychology, University of Lethbridge, Lethbridge, Alberta, T1K 4M4.

The ability of post-ischemic insulin to modify the structural and neurobehavioral consequences of cerebral ischemia was studied. Rats were given intraperitoneal glucose 20 minutes before ischemia induced by 10 min of hypotension and carotid occlusion. Following reperfusion, they were given either insulin (2U/kg, i.p.) or placebo for one week. Sham operated rats (SHAM) were used as a control group. Rats were trained on a learning-set task 1-3 months before and 1-3 months after the brain injury to locate an escape platform in a pool of opacified water. Escape latency and swim pattern were recorded. If a rat deviated from an escape path by 1 to 2 body lengths, it received an error on that trial. Performance in the INS group was significantly better in both escape latency and errors (p<0.05). Thiamine hypophosphatemia predominated in the hippocampus where the GLUT4 group showed 59% mean neuronal loss compared to 8% mean neuronal loss in the INS group (p<0.05). Insulin administration during the early post-ischemic recovery period thus resulted in a significant improvement in performance of behavioral tasks assessing spatial memory, in addition to reducing CA1 hippocampal neuronal necrosis.


Acute increases in plasma catecholamines and myocardial damage occur in a cat model of stroke using middle cerebral artery occlusion (MCAO). Similar changes are seen clinically in patients following stroke. Because of the advantages of using a small animal, we examined autonomic changes in two MCAO models of stroke in the rat. Blood pressure (BP), heart rate (HR) and plasma concentrations of norepinephrine (NE) and epinephrine (E) were measured in 20 male, urethane-anesthetized rats that received one of the following treatments: (i) MCAO only, (ii) MCAO and ipsilateral carotid artery occlusion (MCAO/CCO), (iii) sham occlusions. MCAOs were made immediately distal to the inferior cerebral vein. Arterial blood samples (500 u) for norepinephrine and epinephrine were taken before the occlusions and at 90 and 180 minutes after the occlusions. At the end of the experiment, tetraethylammonium salts were reacted with oxidative enzymes to determine the levels of cerebral autoregulatory changes seen in sham and MCAO/CCO groups significantly declined during the approximately 8 h experiment. However, BP of MCAO rats did not change during the experiment, so that the final BP was significantly higher than in the other two groups. Plasma NE and E concentrations were increased significantly by MCAO compared to pre-occlusion levels and compared to post-occlusion levels in sham and MCAO/CCO groups. These results suggest that focal cerebral ischemia caused by MCAO only in the urethane-anesthetized rat is able to induce the autonomic changes seen in the cat model clinically.

(Supported by the Heart and Stroke Foundation of Ontario)
503.7

TEMPORAL PROFILE OF MEMORY AND HIPPOCAMPAL CA1 CHANGES FOLLOWING 4-VO RAT MODEL OF ISCHEMIA. J. S. Ohr*, S. S. Haghighi (SPON: C. Watts) Division of Neurosurgery, University of Missouri, Columbia, MO 65212

Recently, a new technique has been developed to assess motor pathway integrity using electrical or magnetic pulses applied to the cortex. The evoked motor responses were recorded from the spinal cord, peripheral nerves, or target muscles. To study the effect of hypotension on spinal motor end potentials (SMEs), we subjected twelve cats to graded hypotension from mean arterial pressure (MAP) of 100 mm Hg down to 30 mm Hg. SMEs were continuously recorded from spinal cord using epidural surface electrodes. The exposed precerebral motor cortex was stimulated with 150 microsec, 20 v (max) pulses via AgCl stimulating electrode. The onset latency of SMEs increased from 1.39 ± 0.07 m sec at MAP 100 mm Hg to 2.96 ± 0.07 m sec at MAP 30 mm Hg (p = 0.05). Conduction velocity decreased from 84.76 ± 6.81 meter/second (mean) to 7.81 meter/second (mean) at MAP 30 mm Hg (p = 0.05). The peak to peak amplitude decreased from 7.46 ± 3.84 µv (mean) to 2.12 ± 0.58 µv (mean) at MAP 30 mm Hg (p = 0.05). Decrease in SMEs may not be related to ischemia directly but may be due to decreased perfusion of the spinal cord from the carotid arteries.

503.8


To determine whether functional deficits after stroke can be improved, we have established a behavioral paradigm sensitive to cortical focal ischemia. Focal ischemia is induced in Sprague-Dawley rats (225-275g) by permanent unilateral occlusion of the middle cerebral artery & the ipsilateral common carotid artery (MCAo + CCAo) with a 1 hr temporary occlusion of the contralateral CCA. Twelve days after the MCAo rats are trained in a 2 lever apparatus to press for food reward. On subsequent days, after 8 consecutive correct responses on the initially reinforced lever, the opposite lever is reinforced. Lever reversals continue after 8 consecutive reinforced responses. The session ends after 10 reversals are completed. Rats with focal ischemia [largely localized to the parietal cortex] show an increase in incorrect responses. But, the largest difference between controls and ischemic rats is an increase in perseverative errors (continuing to press a lever even though new reward is provided). We are evaluating the effectiveness of various agents (gangliosides, vitamin E, physostigmine etc) in reducing this functional deficit, and relating these to changes in edema, Na+, K+, Ca++ in ischemic areas.

503.9

EFFECTS OF GRADED HYPOVOLIC HYPOTENSION ON SPINAL MOTOR EVOKED POTENTIALS IN THE CAT. J. J. Dor* S. D. Haghighi (SPON: C. Watts) Division of Neurosurgery, University of Missouri, Columbia, MO 65212

To study the effect of hypotension on spinal motor end potentials (SMEs), we subjected twelve cats to graded hypotension from mean arterial pressure (MAP) of 100 mm Hg down to 30 mm Hg. SMEs were continuously recorded from spinal cord using epidural surface electrodes. The exposed precerebral motor cortex was stimulated with 150 microsec, 20 v (max) pulses via AgCl stimulating electrode. The onset latency of SMEs increased from 1.39 ± 0.07 m sec at MAP 100 mm Hg to 2.96 ± 0.07 m sec at MAP 30 mm Hg (p = 0.05). Conduction velocity decreased from 84.76 ± 6.81 meter/second (mean) to 7.81 meter/second (mean) at MAP 30 mm Hg (p = 0.05). Decrease in SMEs may not be related to ischemia directly but may be due to decreased perfusion of the spinal cord from the carotid arteries.

503.10

EFFECTS OF HEMORRHAGIC SHOCK ON SPINAL AND CORTICAL SOMATOSENSORY EVOKED POTENTIALS IN THE CAT. S. S. Haghighi (SPON: C. Watts) Division of Neurosurgery, Univ. Missouri, Columbia, MO 65202

Somatosensory evoked potentials (SEPs) are being used in the evaluation of spinal and cortical function during surgical procedures. To study the effect of systemic hypotension upon spinal and cortical SEPs, we subjected twelve anesthetized cats to graded hypotension from mean arterial pressure (MAP) of 100 mm Hg down to 30 mm Hg. Spinal and cortical SEPs were recorded after nerve stimulation. The onset latency of spinal SEPs increased from 1.99 ± 0.47 m sec at 100 mm Hg to 2.85 ± 1.04 m sec at 30 mm Hg (p = 0.05); while conduction velocity (CV) decreased from 95.39 ± 16.22 meter/second to 71.97 ± 17.05 (p = 0.05) at the rate of 2.8 meter/second/10 degrees of hypotension change. Cortical SEPs onset latency increased from 8.4 ± 0.07 m sec at 100 mm Hg to 11.1 ± 1.05 m sec at 30 mm Hg (p = 0.05). CV decreased with hypotension from 54.76 ± 7.31 meter/second to 43.19 ± 6.71 at the rate of 1.5 meter/second/10 degrees hypotension change. No cortical SEPs were detectable below 30 mm Hg. Spinal evoked responses were more resistant to profound hypotension and disappeared last. Blood transfusion reversed spinal SEPs first followed by cortical SEPs.

These findings suggest that ischemia associated with profound systemic hypotension can alter evoked responses.

503.11


Amino-substituted 9,10-seco steroids are potent inhibitors of iron-dependent lipid peroxidation as determined in the malondialdehyde (MDA) assay and conjugated diene assay. In addition, they protect animals from the sequelae of head injury damage. In collaboration with Prof. T.Kowana and associates (CBAR, Univ. of Kansas), we report redox potential data which suggests that the amino heterocycle structure of the seco steroids, and their steroisomeric partners (i.e., Lazaroids) may serve as a radical-quenching reducing agent in biological systems.

503.12

INCREASE IN EXTRACELLULAR ASCORBATE DURING FOCAL CEREBRAL ISCHEMIA IN THE RAT MONITORED BY INTRACEREBRAL MICRODIALYSIS. L. Hiller(1), L. Persson(2) and U. Ungerstedt*(3).

Depts. of Clinical Chemistry(1) and Neurosurgery(2), University Hospital, S-751 85 Uppsala, Sweden. Dept. of Pharmacology(3), Karolinska Institute, Stockholm, Sweden.

Apart from its role as a major antioxidant in the brain, ascorbate (AA) is well known to have the paradoxical ability to induce iron-dependent lipid peroxidation. Recently, AA has been implicated as an important modulator of neuro-axial activity. Administration of AA (i.p.) appears to increase neuronal activity in the spinal cord. But, AA has also been implicated in the importance of ascorbate during focal cerebral ischemia. Microdialysis probes (Carnegie Medical AB, Stockholm, Sweden; membrane length 3 mm) were implanted stereotactically into the caudal putamen bilaterally. Dialysis was started 2 hours later using a flow rate of 2 μl/min. Samples were collected in three 30-min fractions before and after the onset of ischemia. Ischemia was induced by middle cerebral artery occlusion on the left side. Compared to the pre-ischemic level and to the contralateral side ischemia was associated with a 6-6 fold increase in ascorbate in dialysates from the left striatum. The level of ascorbate did not change significantly on the contralateral side. We propose that ascorbate may aggravate neuronal injury in the ischemic penumbra and promote lipid peroxidation and/or by an excitotoxic mechanism.
503.13
CONTINUOUS TRANSCRANIAL MONITORING BY LASER DOPPLER VELOCIMETRY DURING TRANSIENT CEREBRAL ISCHEMIA. D.M. Bowden and R.F. Martin. Dynamic changes of cerebral blood flow (CBF) induced by multiple transient bilateral carotid occlusions were studied in the Mongolian gerbil. The data obtained by transcranial measurement of CBF by Laser Doppler Velocimetry (LDV) correlate well with that obtained by intracranial LDV. LDV technique made possible repeated and continuous measurement of these circulatory parameters over long periods of time with minimal attention to the hazards of exposure or infection of the brain. Transcranial LDV provided immediate information about the preischemic normalcy, the completeness of the ischemia and the recovery of blood flow following release of the occlusion which in our studies was a function of the duration of the ischemias. In very short term ischemias, LDV showed that recovery of blood flow was immediate. With longer ischemias CBF recovered partially, followed by slower further increase and formed a hyperemic peak, often with some overshoot. The CBF then slowly fell to a hyperfusion stage, the effects of certain vasoactive drugs on this sequence were studied by LDV and demonstrated the usefulness of LDV to evaluate these substances.

503.15
A comparison was made of the efficacy of the 21-amino steroid oxygen radical scavenger and lipid peroxidation inhibitor U74006F to antagonize post-ischemic degeneration in the hippocampal CA3 region of the Mongolian gerbil after either a 10 min. bilateral carotid occlusion (BCO) of the carotid arteries or a 3 hr. occlusion of one carotid artery (UCO). At 1 week after the 10 min. BCO, pronounced (75-85%) selective CA3 cell loss was apparent. Pretreatment (30 min.) with U74006F over a wide range of i.p. doses (1, 10, or 30 mg/kg) plus a second dose at 2 hrs. after BCO had no effect on the CA3 cell loss. In the 3 hr. UCO model, a similar degree of CA3 cell loss was observed after only 0.5% of the 10 min. BCO dose. In addition to diffuse degeneration throughout the ipsilateral hemisphere. In this model, i.p. pretreatment with U74006F (10 mg/kg) plus a second dose at the end of the 3 hr. UCO significantly reduced CA3 damage as well as the cell loss in other brain areas (e.g., cortex). These results suggest that the mechanism of CA3 degeneration may differ between ischemia models with oxygen radical-induced lipid peroxidation perhaps being more involved in the 3 hr. UCO model.

504.1

The Macaque Human Glossary of Neuroanatomical Nomenclature allows the user to identify the brain structure to which any generally accepted English or Latin neuroanatomical name applies. When the user enters a structure name into the computer, the computer responds with a list of all accepted synonyms and with references to one or more macaque and human brain atlases in which the structure is defined or illustrated. The computer shows where in the hierarchy of structures and substructures the named structure lies, i.e., button clicks take the user up the limb of suprastructures or out the branches of substructures to indicate how the named structure relates to other parts of the brain. The glossary includes a total of 750 structure names and more than 2300 synonyms. The hierarchy is based on the human Nomina Anatomica with extensions based on several macaque and human brain atlases. Supported by NIH grant RR00166 to the University of Washington.

504.2
We determined an encephalization quotient (EQ) of 0.275 for T. manatus, based on direct measurements of brain mass (avg-364g) and body mass (avg-756kg) in 13 specimens. This is among the lowest values for all mammals and is comparable to EQ estimates for other Sirenian species. However, when our data are adjusted for metabolic rate, actual brain weight is 1.5 times larger than predicted. The unique aquatic herbivorous lifestyle of the Sirenia has likely been a major determinant of large body size and low metabolic rate. We suggest that early in Sirenia history brain-body size allometry was uncoupled, permitting selection for an increase in body size without a corresponding change in relative brain size. Low metabolic rate may also be a factor in constraining brain size in manatees.

Gyration of the cerebral cortex, usually positively correlated with absolute brain size, is strikingly absent in these brains. However, morphometric data indicate that the telencephalon comprises 71% of total brain volume in T. manatus. This is comparable to values for prosimians and monkeys and much larger than values for insectivores and bats. Likewise, manatee cerebral cortex is well laminated and of robust cellular density. A retarded growth curve for the brain may explain the isensephalic condition. In any case, internal structural complexity of the brain appears to have been unaffected by the size restrictions implied by low EQ, or by the isensephalic.

Supported by grant BSR-03687 from NSF, and cooperation of US Fish and Wildlife Service, Florida DNR, and Sea World of Florida, Inc.
504.3 NEURONAL ORGANIZATION AND MICROCIRCUITRY OF LAYERS I AND II IN VISUAL CORTEX OF DOLPHINS (STENELLA COERULEOALBA AND TURRIOPS TRUNCATUS), P.J. Morgan and I.L. Glezer. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, and CUNY Medical School, New York, NY 10031.

The neuronal composition and microcircuitry of layers I and II of the dolphin visual cortex were analyzed. We have found that the cortical layers are similar to those seen in most conservative euhameran mammals such as basal Insectivora. Layer I is most predominant and occupies nearly the entire cortical area. Layer II is characterized by an extremely dense concentration of neurons. By using rapid Golgi impregnation, we were able to analyze the horizontal and dendritic spectrum of layer II neurons. The axonal and dendritic composition of layer I in the dolphin neocortex. The major neuronal types seen in layer II are transsitional pyramidal with slender apical dendrites extending widely into layer I and, secondarily, other transitional pyramids with two apical dendrites which ascend widely into layer I ("extraverted" neurons). The neuronal organization of layers I and II in the dolphin neocortex provides the preliminary basis for our hypothesis stating that these layers are the main afferent input layers from subcortex and for associative fibers, providing intracortical connections. (Supp. by NSF grants RNS 84-5732, 87-42032, and 83-442-2490, 4-67-204 and the Osborn Laboratories of the New York Aquarium of New York Zoological Society).


Objective Potentiation of Neuroleptic-Induced Extrapyramidal Function. The present study investigated the effect of nicotine on haloperidol-induced catalepsy in an effort to elucidate the role of nicotine in modulating the function of the extrapyramidal system. Recent studies in our laboratory revealed that nicotine produces a marked decrease in the severity of haloperidol-induced catalepsy. In the present study, we investigated the effect of nicotine on haloperidol-induced catalepsy in an effort to elucidate the role of nicotine in modulating the function of the extrapyramidal system.


The degree of cortical folding in primates varies from lissencephalic primatians to the highly convoluted human brain and can be analyzed in serial sections by measuring the lengths of the complete outer cortical contours and those parts which are superficially exposed. Ratios of outer cortical outline to that of the superficially exposed part of the cortex. This ratio was used to determine the degree of folding in primates ranging in age from birth to adulthood. The degree of cortical folding was assessed using rapid Golgi technique. Several features characterized the cortical folding in primates. Within Homo, GI is on the order of 100 fold. It has been proposed that cannabinoids may be useful to increase the efficacy of NIC (0.1 mg/kg gavage). Another feature is the presence of extremely large numbers of synapses on passage which are especially abundant in layer I. Multiple vesicle types are seen in nearly all synaptocystons. Most of the ascending collaterals of subcortical and intracortical axons reach layer I in the dolphin convexity near the periphery of the gyri. The neuronal organization of layers I and II in the dolphin neocortex provides the preliminary basis for our hypothesis stating that these layers are the main afferent input layers from subcortex and for associative fibers, providing intracortical connections. (Supp. by NSF grants RNS 84-5732, 87-42032, and 83-442-2490, 4-67-204 and the Osborn Laboratories of the New York Aquarium of New York Zoological Society).

504.6 THE OTOGRAPHY OF CORTICAL FOLDING IN THE HUMAN BRAIN. E. Ackermann, and J. T. Bongard. Anatomy Department, H.S.B., Bethesda, MD, and Anatomy Department, H.S.B., Bethesda, MD.

Cortical folding in serially sectioned human brains was examined by measuring the lengths of the total cortical contour and that of the outer cortical contours and those parts which are superficially exposed. The ratio of outer cortical contour to superficially exposed part of the cortex was used to determine the degree of folding in primates ranging in age from birth to adulthood. The degree of cortical folding was assessed using rapid Golgi technique. Several features characterized the cortical folding in primates. Within Homo, GI is on the order of 100 fold. It has been proposed that cannabinoids may be useful to increase the efficacy of NIC (0.1 mg/kg gavage). Another feature is the presence of extremely large numbers of synapses on passage which are especially abundant in layer I. Multiple vesicle types are seen in nearly all synaptocystons. Most of the ascending collaterals of subcortical and intracortical axons reach layer I in the dolphin convexity near the periphery of the gyri. The neuronal organization of layers I and II in the dolphin neocortex provides the preliminary basis for our hypothesis stating that these layers are the main afferent input layers from subcortex and for associative fibers, providing intracortical connections. (Supp. by NSF grants RNS 84-5732, 87-42032, and 83-442-2490, 4-67-204 and the Osborn Laboratories of the New York Aquarium of New York Zoological Society).
505.3 AUTOTOMY INDUCED BY SUBCUTANEOUS INJECTION OF HYPERTOCRINE SALINE IN THE RAT. T. Komori* and H. Nakazato*.

The behavior of autotomy induced by saline injection was studied in 30 Wistar rats (150-450 g). A standard dose of 0.15 ml saline injected subcutaneously, 2 cm from the tip of the tail was used. For the first 2 h, the rats licked the site of injection and tail in a manner similar to that of the normal. After 8 ± 3.3 weeks, the onset was 2.26 ± 1.52 weeks. A second application of 0.15 ml saline in animals showing autotomy, resulted in autotomy in 40% of the cases. After autotomy, no signs of phantoms limb pain were seen for a period of 1 year.

The data presented suggest that peripheral chronic pain may initiate autotomy followed by signs of ischaemia and necrosis which may lead to amputation.


505.5 DECREASED IL-2 PRODUCTION, INCREASED IL-2 RECEPTORS, AND INCREASED IL-2 PRODUCTION IN SCHIZOPHRENIA. H. A. Marshall, S.C. Olson*, J.A. Coffman and S.B. Schwartzkopf*.

Studies from 18 normals were processed to calculate the mean and standard deviation for each resampled voxel. Twenty DSM-III-R diagnosed schizophrenic patients (SC) were evaluated for regional statistical differences from the normal group. Quantitative differences between frontal and temporal regions were confirmed. This method classified chronic paranoid schizophrenic patients (CPS) were evaluated for regional statistical differences from the normal.

The basis of autotomy behavior is still unknown (Rabin, A.G. et al., Pain, 21:117-128). The phenomenon was studied in 30 Wistar rats (150-450 g). A standard dose of 0.15 ml saline injected subcutaneously, 2 cm from the tip of the tail was used. For the first 2 h, the rats licked the site of injection and tail in a manner similar to that of the normal. After 8 ± 3.3 weeks, the onset was 2.26 ± 1.52 weeks. A second application of 0.15 ml saline in animals showing autotomy, resulted in autotomy in 40% of the cases. After autotomy, no signs of phantoms limb pain were seen for a period of 1 year.

The data presented suggest that peripheral chronic pain may initiate autotomy followed by signs of ischaemia and necrosis which may lead to amputation.


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Tourette’s Syndrome is a complex disorder of motor and verbal tics. While the drugs of choice for this disorder are dopamine receptor blockers, such as haloperidol, some patients show only marginal response. Furthermore, these drugs can lead to sedation, exacerbation of learning difficulties, and possible tardive dyskinesia. For these reasons, an agent that potentiates the effects of neuroleptics could allow lower doses of neuroleptics and reduction in side effects.

In animals it has been shown that nicotine markedly potentiates the behavioral effects of haloperidol (Moss et al., and Manderscheid et al. this issue). Nicotine gum was given to patients with Tourette’s Syndrome who showed only moderate responses to haloperidol alone. A marked reduction in the intensity of tics and an increase in attention and comprehension was seen in six of the seven cases. In some cases the tics stopped completely. This effect started after 15 minutes, lasting about one hour. Side effects including bitter taste and gastric distress did not occur.

Nicotine, gum may prove useful in treating neuroleptic-responsive disorders.


Forty schizophrenic patients (by DSM III-R) were interviewed with their parents for first degree family history (FH) of psychosis and for various perinatal brain injuries. MRI scans were obtained (TR=1500 MS, TI=800 MS). Midsagittal cerebral, frontal, and ventricular areas were measured. No significant correlations were found between perinatal events and MRI variables. On the other hand, FH correlated significantly with smaller cranial size (p< .06) and with a strong trend for smaller cerebral and frontal size as well. The results indicate that developmental factors may be associated with perinatal brain injury rather than genetic loading.

40 schizophrenic patients (by DSM III-R) were interviewed with their parents for first degree family history (FH) of psychosis and for various perinatal brain injuries. MRI scans were obtained (TR=1500 MS, TI=800 MS). Midsagittal cerebral, frontal, and ventricular areas were measured. No significant correlations were found between perinatal events and MRI variables. On the other hand, FH correlated significantly with smaller cranial size (p< .06) and with a strong trend for smaller cerebral and frontal size as well. The results indicate that developmental factors may be associated with perinatal brain injury rather than genetic loading.


The sections were stained with the Holzer technique (Rabin, A.G. et al., Pain, 21:117-128). The phenomenon was studied in 30 Wistar rats (150-450 g). A standard dose of 0.15 ml saline injected subcutaneously, 2 cm from the tip of the tail was used. For the first 2 h, the rats licked the site of injection and tail in a manner similar to that of the normal. After 8 ± 3.3 weeks, the onset was 2.26 ± 1.52 weeks. A second application of 0.15 ml saline in animals showing autotomy, resulted in autotomy in 40% of the cases. After autotomy, no signs of phantoms limb pain were seen for a period of 1 year.

The data presented suggest that peripheral chronic pain may initiate autotomy followed by signs of ischaemia and necrosis which may lead to amputation.


Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

Recently several authors have claimed prominent cytoarchitectural abnormalities in the entorhinal cortex of both Alzheimer’s disease (AD) and schizophrenic patients (SC). In the present study, we have attempted to corroborate the presence of such a lesion in both AD and SC patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 control patients.

Several previous studies have demonstrated differences in regional brain density (as derived from CT scan attenuation values) between patients with schizophrenia and normal controls. Interpretation of these studies has been hindered by methodological shortcomings such as failure to control for head size, scanning calibration differences, and other confounding variables. The present study offered methodological advances over earlier studies by controlling for head size and normalizing the attenuation values for each scan to an internal standard. CT attenuation values in multiple brain regions in 20 patients with chronic schizophrenia were compared with those of 20 age and sex matched controls. No significant differences in regional attenuation values emerged between the schizophrenics and normal controls. These results confirm the importance of controlling for artifacts in analysis of CT scan attenuation values and raise questions about the validity of regional CT attenuation values in detecting subtle anatomical abnormalities in schizophrenia.


Unipolar depressed patients were treated with imipramine (IMI) for 4-13 wks. Bmax values were increased by 33% after 4 wks but later returned to baseline even though the patients remained euthymic. These changes were independent of plasma drug levels and were not due to drug carryover. IMI treatment did not alter the Kd with no change in Bmax in vitro. Post-treatment Bmax and plasma cortisol levels were inversely related but were uncorrelated to baseline and there was no difference between pre- and post-treatment cortisol levels. Treatment response was not significantly correlated with either the Bmax or plasma cortisol changes, although relatively poor responses were associated with extreme values (high and low) of pretreatment Bmax. The results suggest that chronic IMI treatment results in transient increases in Bmax through a cortisol dependent mechanism. Since these changes were not related to therapeutic response, the results further support the limited usefulness of 4-13 week drug trials in the assessment of the state dependence of platelet binding.

Responses to clonidine in acute and remitted depressed patients.

Larry J. Siever, M.D., Emil F. Coccaro, M.D., Peter Knott, Ph.D., Ph.D., Ren-Yi Yang, M.D., Steven Gabrieli, Ph.D., and David J. Evans, M.D.

Bronx VA Medical Center, Bronx, NY and Mount Sinai School of Medicine, New York, NY.

Clonidine, an alpha-2-adrenergic agonist which results in increases in plasma growth hormone (GH) and decreases in plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), was administered to 28 acute depressed patients, 15 remitted depressed patients, and 12 normal controls. All subjects were free of medical illness, drug-free for at least two weeks, and on a low monoamine diet for three days prior to testing. A reduced growth hormone response to clonidine (45 mg/ml) was found in 68% (19/28) of the acute depressed patients and in 67% (10/15) of the remitted depressed patients as compared to 25% (3/12) of the age- and sex-matched controls (acute normal, remitted normal, Fisher's Exact, p<0.05). All patients initially blunted in the acute state remained blunted in the remitted state. Acute depressed patients demonstrated lower absolute (0.1 ± 0.5) or percent decrease (0.9 ± 10.7%) in plasma MHPG compared to normal controls (absoluto 0.4 ± 0.6, percent: 8.4 ± 14.8) (t-test, p<0.05), while remitted patients were similar to controls. These results suggest that the GH response to clonidine is blunted in depressed patients regardless of state, while the reduced MHPG response to clonidine is state-dependent.

BRAIN METABOLISM DURING AUDITORY HALLUCINATIONS. J.M. Cleftorn, Dept. of Psychiatry, McMaster University, Hamilton, Ontario Canada L8N 3S5.

These studies attempt to locate regions of the brain associated with auditory hallucinations by means of positron emission tomography with [18F] fluoro-deoxy-glucose. Data on two samples will be reported. 1: first episode drug naive psychiatric patients (N=14) with a history of 11 hallucinations were hallucinating and 6 were not hallucinating during the glucose uptake period prior to the scan. 11: chronic patients (N=19) medicated for an average of 7.4 years, in whom auditory hallucinations were still present in 9 and had disappeared in 10.

Hallucinating (H) and nonhallucinating (NonH) groups did not differ from each other in the regions in which glucose metabolism was measured: prefrontal and orbitofrontal cortex, parietal cortex, superior temporal and Wernicke's cortex. Broca's and auditory cortex metabolism was also similar in H and NonH groups.

However, correlations between some brain regions characterized both H groups and were not observed in the NonH groups: right hemisphere regions homologous for Broca's and auditory areas r = +0.81 (p<0.01) and right frontal and parietal r = +0.80 (p<0.01). In the drug free H group the right sided homologous regions for Broca's and Wernicke's cortex are significant.

These studies suggest that right hemisphere regions homologous to language areas on the left are coupled during auditory hallucinations.


Patients with cerebrovascular lesions in the posterior circulation (PC) territory (n=37) were compared with patients having middle cerebral artery (MCA) (n=42) strokes for the presence of mood disorders. While both groups showed a similar profile of clinical symptoms of depression during the acute evaluation in-hospital, patients with PC lesions involving the brainstem and/or cerebellum demonstrated a significantly lower frequency of depression (27%) than patients with MCA lesions (48%) or patients with PC lesions involving the left cerebral hemisphere (100%). Moreover, at two-year follow-up, depression following brainstem and/or cerebellar injuries was significantly shorter in duration than depression following MCA lesions (mean Present State Exam depression scores from in-hospital to 2 years follow-up were significantly lower for the brainstem/cerebellar group than the MCA patients with in-hospital depression over the same period, repeated measures ANOVA group by time F(1,39) = 13.3, p<0.001). These differences in the frequency and duration of depression following brainstem/cerebellar as compared with MCA lesions were not explained by differences in lesion volume, physical impairment, cognitive deficits or quality of social support. They suggest that PC and MCA-induced depression may have different etiologies.

SECONDARY vs IDIOPATHIC MANIA. B. Roffi*, R. C. Young, G. Kierman* (SPON: M. Rusa). The New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605.

"Secondary" or symptomatic manic syndromes - those presenting in patients with associated medical disorders or drugs implicated as etiologic - have not been studied systematically. We retrospectively studied patients admitted over a three year period to two university psychiatric hospitals. Patients with a DSM III diagnosis of organic affective disorders, manic (N = 212) and with bipolar disorder, manic (N = 212) were contrasted using a computerized data base. Organic patients were older at index hospitalization (46.8 years ± 15.1 years vs. 43.7 years ± 18.4 years; p<0.14), were older at first psychiatric hospitalization (45.8 years ± 22.8 years vs. 31.0 years ± 15.1 years; p<0.01), and had more severe hospitalizations (1.3 ± 0.9 vs. 2.3 ± 2.8; p<0.001). They had longer duration of index hospitalization (54.6 days ± 29.5 days vs. 25.4 days ± 29.7 days; p<0.01), lower Global Assessment Scale (GAS) scores at discharge (41.3 ± 9.3 vs. 56.0 ± 13.8; p<0.001), and less improvement in GAS scores during hospitalization (10.5 ± 10.4 vs. 22.5 ± 16.7; p<0.01). These preliminary findings suggest that "secondary mania" is a syndrome with later age at onset and poorer treatment outcome.
505.15

ANTIDEPRESSANTS AND LATE LIFE MANIA. H. Jain* and R.C. Young. The Permanente Medical Center, Westchester Division, White Plains, New York 10605.

Manic episodes can occur for the first time in late life. Innic factors and pathophysiology may differ from those in patients in whom mania occurs first in early life. Charts of geriatric (age > 65) depressed psychiatric inpatients (n=440) who met DSM III criteria for bipolar disorder, manic, were reviewed. The median age at onset of a first manic episode was 50.6 years with range 18 to 81 years. Seven (16%) of the patients with late age at onset of first manic episode had been treated with anti-depressants. These patients were older and had a more florid course of illness than those who had not been treated with anti-depressants. These findings suggest that in geriatric bipolar patients with later age at onset of first manic episode are more vulnerable to induction of mania by antidepressant drugs than are geriatric patients with earlier first occurrence of mania.

505.16


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505.17

CAFFEINE SUPER-SENSITIVITY IN PANIC DISORDERS. E.M. DeMet, Dept. Psychiatry, Univ. of Calif., Irvine, CA 92717.

Panic disorder (PD) patients are frequently sensitive to the anxiogenic effects of caffeine. These effects may be due to an antagonistic action on adenosine receptors which attenuate excitatory neurotransmission. The present study produced a novel measure of adenosine receptor sensitivity and compares results obtained from normal controls with those from patients with PD and post-traumatic stress (PTSD). The test is based on a known action of adenosine receptors to potentiate the ability to taste quinine sulfate. Quinine taste thresholds were determined by a forced choice discrimination test from water standards. Adenosine receptor sensitivity was quantitated by comparing thresholds in the presence and absence of 10uM caffeine. Thresholds in the presence of caffeine were similar in the 3 subject groups. In contrast, PD patients had elevated baseline thresholds and larger difference scores than did controls or PTSD patients. The results are discussed in the context of a model whereby adenosine receptors upregulate in an attempt to control excitatory transmitter release, but this same mechanism confers caffeine supersensitivity.

505.18


Good evidence from many laboratories supports the belief that amphetamine (AMP) and methylphenidate (MPH) often produce remarkable ameliorative effects in children with ADHD, actions believed to occur via central catecholaminergic mechanisms. Despite their similarities, AMP and MPH may affect different catecholaminergic mechanisms. We employed a double-blind protocol to investigate 26 children with ADHD (24 boys, 2 girls) ages 5-14 years. Children received either AMP (.25 mg/kg, 12 children) or MPH (0.1 mg/kg, 12 children) or placebo (n=26) and plasma MHPG and HVA were sampled:

<table>
<thead>
<tr>
<th>Hours after stimulus</th>
<th>Placebo</th>
<th>AMP</th>
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<tr>
<td>0</td>
<td>5.18±.31</td>
<td>5.74±.41</td>
<td>5.74±.41</td>
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<tr>
<td>4</td>
<td>5.18±.31</td>
<td>5.74±.41</td>
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These findings suggest that despite their clinical similarity, d-amphetamine and methylphenidate differ in their effects on brain catecholaminergic systems with d-amphetamine influencing brain noradrenergic mechanisms, perhaps by reducing turnover of brain norepinephrine.

505.19

PRODUCTION OF NEGLECT IN RATS WITH UNLATERAL ABLATION OF VENTROLATERAL ORBITAL CORTEX. V. Hon* U.V. Surawicz, and R.L. Reep, Dept. of Psych., Univ. of Calif., Irvine, CA, 92717.

Neglect in mance and malkins most often results from damage to cortical areas, including parietal cortex. Neglect in rodents with unilateral destruction of medial prefrontal cortical area (vLO), a rodent analog of area 9 in primates. As with area 9, vLO has extensive connections with neocortical and subcortical structures, including the hypothalamus. The current study evaluated the effects of lesions involving the orbitofrontal cortex just lateral to vLO (sham) (n=15) were tested for a minimum of four weeks on the following: 1) total neglect based on degree of orientation to the ipsilesional tab first when adhesive tabs were attached to each wrist; 3) motor behavioral deficits, 23 BXSB mice were given a series of behavioral tests. Eight had ectopias in the frontal motor region (5M, 3F) while 15 did not (BM, 7F). On Collins’ paw reaching test ectopic mice used their left paws almost exclusively while ectopic females were equally strongly biased rightward. The normal males and females were scattered throughout (p<0.4). Water escape learning is a simple spatial task requiring the animal to swim to a submerged platform. Mice with ectopic lesions were faster, averaged 28.1 sec over five trials while mice with normal brains took 52.8 sec (p<0.05). In a non-spatial behavioral test, task, the learning difference was significant (p<0.05). M EOF mice made more correct choices than those with normal brains (p<0.05), while the female groups did not differ.

Since ectopic animals swim faster in the water escape task but slower in the discrimination learning task, the learning difference may be due to motor factors that would influence speed of swimming. Some feature distinguishing the two tasks--such as spatial vs non-spatial, or choice vs no-choice--may appear to be associated with the presence of ectopias. In addition, left pawedness, maleness, and presence of ectopias were associated on the discrimination learning task.
THE CONE ELECTRODE: A LONG-TERM ELECTRODE THAT RECORDS FROM NEURITES. R.G. Shanks, Bioengineering Center, Georgia Institute of Technology, Atlanta, GA 30332.

A standard wire recording technique is combined with neurite growth into a piece of sciatric nerve to produce an electrode that records for months. The electrode is made by fixing a teflon insulated 3 mil gold wire to the inside of a 1.8 mm glass cone with a diameter of less than 200 microns at one end. Before Implantation in deep layers of rat cortex, 5 to 10 fibers from the rat's sciatric nerve are allowed to grow into the cone. The pin on the other end of the wire is cemented to the rat's skull.

For a few days after Implantation no activity is evident. As the cone activity builds up, first as background, then as single units. At week 4, many units appear so that multi-unit activity over 100 µVs in amplitude is recorded. When Implantated into the vibrissa area of cortex, many vibrissa evoked multi-unit activity, but from week 4 onwards, only 2 to 3 adjacent vibrissa evoke multi-unit responses. This suggests that a connection (a nerve) must be made between the cone electrode and functional areas of cortex. Histology at 3 months when still recording shows tissue growing into the cone holding it firmly in place. Retrograde dye labelling via the corticospinal tract shows neurites in the cone.

The cone electrode is expected to connect the central nervous system with augmentative devices in patients with severe communicative disorders.

BEHAVIORAL PHARMACOLOGY: MISCELLANEOUS

506.1


FG 7142 (N-methyl-β-carboline-carboxamide), a partial inverse agonist at the benzodiazepine receptor, produces both anxiogenic and proconvulsant effects. In the discriminative stimulus (D.S.) paradigm, FG 7142 produces drug-appropriate responding in SMC and pentyleneetetrazole trained rats. D.S. control has been established with FG 712 and generalizes to red and stressful environment manipulations that was occasionally electric (0.5mA), electric stimulation via the midbrain periaqueductal gray (PAG). The midbrain periaqueductal gray (PAG) has been implicated in the initiation and regulation of aggressive behavior in the cat. Since the PAG is rich in GABA receptors, we examined the role of this putative transmitter in the modulation of effective defense (AD) and quiet biting attack behavior (QBA) elicited by electrical stimulation of the PAG.

Canula-electrodes were employed for electrical stimulation as well as for microinjections of a GABA agonist (muscimol: 3, 12, 23, and 44 pmol/0.25µl) and GABA antagonist (bicuculline: 22 µmol/0.25µl). After establishing pre-drug response threshold values for AD and QBA, these drugs were microinjected into the PAG sites from which these responses were elicited. Microinjections of muscimol (12-44 pmol) significantly suppressed AD in a dose and time dependent manner. Pretreatment with bicuculline blocked the suppressive effects of muscimol (23 pmol) upon AD. In contrast, this dose of muscimol failed to alter the response threshold for QBA. Microinjections of vehicle alone (0.25 µl of saline, pH=7.4) did not modify the thresholds for either of these responses.

These results indicate that, at the level of the PAG, GABAergic mechanisms are selectively involved in the regulation of AD behavior in the cat. [Supported by NIH Grant NS07941-19].

506.2

GABA-ERGIC MODULATION OF FELINE AGGRESSION ELICITED FROM THE MIDBRAIN PERIAQUEDUCTAL GRAY. M.B. Shaikh and A. Slezg, Dept. of Neurosciences, UMDNJ, Newark, N.J. 07103.

The possible antagonism of ethanol's behavioral effects by the beta-carboline Zk93426 as compared to the imidazo-beta-carbolines Ro15-1788 and Ro15-4513, were studied in confrontations between resident and intruder rats as well as in interactions in group-housed squirrel monkeys. Quantitative ethological methods permitted the measurement of drug effects on elements of aggressive, defensive, submissive, and social behavior as well as on motic activities. The low doses of ethanol (0.1, 0.3 mg/kg) enhanced frequency of agonistic behavior, while higher doses (1.0, 3.0 mg/kg) reduced their occurrence in both species. Zk 93426 (3.0 mg/kg), Ro15-1788 (10.0 mg/kg) and Ro15-4513 (1.0 mg/kg) did not antagonize the suppressive effects of ethanol in rats. Ro15-4513 and Ro15-1788 potentiated ethanol's sedative effects, and reduced agonistic behavior; Ro15-1788 increased feeding, and Ro15-4513 induced tremors and seizures in squirrel monkeys when administered alone. However, Ro15-4513 reduced ethanol-induced staggering. These results indicate some of these substances completely block the biphasic effects of ethanol; yet the effects of Ro15-4513 on staggering behavior show some promise for identifying one element in the multiple mechanisms of action of ethanol.

506.3


The present study examined the acute effects of the anxiolytics diazepam (DZ) and phenobarbital (PhB), the A-1 selective adenosine agonist 8-2'-deoxycytidine adenosine (1-PIA), and the A-2 selective adenosine agonist N-Methyl-β-carboxy amino adenosine (NECA) on behavior in the 5-N-ethylcarboxamido adenosine (NECA) pretreatment altered the effects of DZ. In contrast, pretreatment with 1-PIA, but not NECA, reduced the anti-conflict effects of PhB. These data suggest that (1) neither A-1 nor A-2 adenosine receptor activation affects basal behavior in the GSD paradigm and (2) PhB, but not DZ, anti-conflict responses may result from interactions with A-1 adenosine receptors. (RE #42501-01; protocol conforms with NIH guidelines)

506.4

ALCOHOL-BENZODIAZEPINE RECEPTOR INTERACTIONS: AGGRESSIVE BEHAVIOR AND MOTOR ACTIVITY IN RATS AND SQUIRREL MONKEYS. E. Weerts*, W. Tornatzky* and K.A. Miczek, Dept. of Psychology, Tufts University, Medford, MA 02155.

The possible antagonism of ethanol's behavioral effects by the beta-carboline Zk93426 as compared to the imidazo-benzodiazepines Ro15-1788 and Ro15-4513, were studied in confrontations between resident and intruder rats as well as in interactions in group-housed squirrel monkeys. Quantitative ethological methods permitted the measurement of drug effects on elements of aggressive, defensive, submissive, and social behavior as well as on motic activities. The low doses of ethanol (0.1, 0.3 mg/kg) enhanced frequency of agonistic behavior, while higher doses (1.0, 3.0 mg/kg) reduced their occurrence in both species. Zk 93426 (3.0 mg/kg), Ro15-1788 (10.0 mg/kg) and Ro15-4513 (1.0 mg/kg) did not antagonize the suppressive effects of ethanol in rats. Ro15-4513 and Ro15-1788 potentiated ethanol's sedative effects, and reduced agonistic behavior; Ro15-1788 increased feeding, and Ro15-4513 induced tremors and seizures in squirrel monkeys when administered alone. However, Ro15-4513 reduced ethanol-induced staggering. These results indicate some of these substances completely block the biphasic effects of ethanol; yet the effects of Ro15-4513 on staggering behavior show some promise for identifying one element in the multiple mechanisms of action of ethanol.
which mediate locomotor behavior are GABAergic and project to the ventral pallidum/substantia innominata (VP/SI) region. The VP/SI region also contains a high density of enkephalin (ENK)-I.R. This study examined whether GABAergic or enkephalinergic neurons located in the VP/SI affects locomotor activity in rats. Intra-V P injection of either picrotoxin or bicuculline produced a dose-dependent increase in photocell counts with a minimum effect dose of 0.03 μg/side. To evaluate a possible interaction between GABA and ENK in the VP/SI, rats were pretreated with 0.1 μg/side (intra-V P) of the mu opioid antagonist naloxone followed by injection of picrotoxin (0.1 μg/side). Naloxone failed to attenuate the picrotoxin-induced hyperactivity. We also investigated the effect of intra-V P injection of the mu opioid agonist DAGO (Met-Enk analog). DAGO produced a dose-dependent increase in locomotor activity with a minimum effect dose of 0.01 nmol/side. Pretreatment with 1.0 mg/kg i.p. of naloxone reversed the effect of DAGO (0.03 nmol/side; intra-V P). These data indicate that inhibition of GABAergic transmission within the VP/SI produces a motor stimulant response which appears to be independent of the VP/SI. Furthermore, stimulation of enkephalinergic transmission in the VP/SI causes increased locomotor activity.


Based upon differences in open field and conflict behaviors, the MH/Bar and MR/Har rat strains have been proposed as a genetically-based "animal model" for the study of emotionality and/or anxiety. The present study compared the MH/Bar and MR/Har rat strains in the Defensive Burying (DB) paradigm described by Tresl (Br. Res. Bull. 19:401-404, 1987). After four daily habituation sessions, female rats were placed in the DB chamber singly. Subjects received a 3 mA shock upon contact with a wire-wrapped prod and were observed for burying behavior (movement of the bedding material toward or over the prod) for 15 minutes post-shock. Although MH/Bar rats tended to initiate burying behavior earlier and exhibited a longer duration of burying than the MR/Har rats, there were no differences in the frequency of subjects exhibiting burying behavior between MH/Bar (12/14) and MR/Har (11/13) rats. Thus, although they differ dramatically in open field and conflict behaviors, the Maudsley rat strains do not differ in measures of DB behavior. The (M#42501-01) protocol conforms to NIH Guidelines.

THREE-CHOICE DRUG DISCRIMINATION OF AN UNDIMENSIONAL "ANXIOGENIC/ANXIOLYTIC" CONTINUUM. D.V. Goodwin*, and F.A. Holloway. (SPONSOR: M.D. Christensen) Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

To lend support to our previous suggestion that the intercerebral states induced by chloridiazepoxide (CDP) and pentylenetetrazol (PTZ) lie at polar ends along a single affective scale (Michaels et al., Psychopharmacology, in press, 1988), 6 Sprague-Dawley rats were trained in a 3-choice drug discrimination task utilizing CDP (5 mg/kg), saline (SAL), and PTZ (11 mg/kg) (Camil). Average sessions to 90% criterion was 102 ± 5. Data from one representative subject is shown below. Generalization tests resulted in pharmacologically specific, quantitative, unidimensional functions.

ENHANCEMENT OF OPIOID CATELEPTIC RESPONSE BY CORTICAL DEAFFERENTATION OR INTRASTRIATAL INJECTION OF NMDA-RECEPTOR ANTAGONISTS. S. Consolo*, G.L. Forloni*, H. Ladinsky and E. Palazzi*. Mario Negri Institute, Milan, Italy.

The cataleptic activity of morphine and methadone was markedly potentiated in decorticated rats with no changes in the onset or duration of action. Enhancement of opioid catalepsy was not due to changes in the availability of the drugs in the brain. The potentiation of methadone induced catalepsy in decorticates, 2) oxotremorine (i.s.) reverses the involvement of the striatum in the phenomenon. That the striatum plays a critical role in opioid-induced catalepsy was substantiated by the findings that: 1) naloxone (i.s.) prevents the potentiation of catalepsy induced by methadone and morphine in decorticates, 2) met-enkephalin (i.s.) elicits the enhancement of opioid catalepsy in decorticates. In conclusion, evidence is given that the corticostriatal pathway exerts an inhibitory effect upon narcotic-induced cataleptic behavior. (Supported by AFOSR-B-0399).

A large body of data suggests that the excitatory amino acids glutamate and/or aspartate may be the neurotransmitters released by the auditory nerve at the cochlear nucleus. We are using the acoustic startle reflex to behaviorally examine the role of excitatory amino acids and their receptors in auditory transmission at the level of the ventral cochlear nucleus (VCN), the first synaptic relay in the neural pathway mediating the startle reflex.

Rats were cannulated in one VCN and received an electrolytic lesion of the contralateral VCN. Because this surgical procedure itself decreases startle amplitude, at animals were prenatfed with the phosphodiesterase inhibitor rolipram (1 mg/kg), g which has been shown to elevate startle. Ten minutes later, rats were infused with either 5, 25, or 50 nmols of gamma-D-glutamylglycine, a potent non-specific excitatory amino acid antagonist, or with artificial cerebrospinal fluid (ACSF). Startle elicited by 50B, 50 ncac noise bursts was measured over the next 40 minutes.

ACSF had no effect on startle. In contrast, gamma-D-glutamylglycine caused a dramatic, dose-dependent depression of the startle reflex, with the highest dose producing a complete blockade of startle. These results support the idea that excitatory amino acids are involved at the level of the ventral cochlear nucleus, and illustrate that the acoustic startle reflex is a sensitive behavioral assay of VCN receptor function. Current work utilizing more specific NMAA and non-NMMA antagonists will attempt to characterize the amino acid receptor subtype(s) in the VCN mediating the acoustic startle reflex.

506.13 EFFECTS OF CHRONIC NIMIDINE TREATMENT ON BEHAVIOR OF OLD RATS. A. Friedl*, T. Schenkouen, M. Klein* and J. Tabor*. (SPONSOR: K.A. Braun). Neurobiology Department, Trojan Pharmaceuticals, Neurobiology Ring 1, D-5000 Kln 80, F.R.G.

Growing evidence suggests an important role in aging processes in the brain to a dysfunction of the Ca"2+-homeostasis in neurons. Compounds which interfere with the Ca"2+ channel such as calmodulin, therefore possess a therapeutic potential in age related brain disorders. Nimidinium, a Ca2+-blocker of the dihydropyridine type, was investigated after subchronic and chronic treatment for its ability to improve behavioral and emotional correlates such as impaired learning and memory capacity, reduced open field activity and social behavior, impaired motor coordination etc. Nimidinium treatment for one week improved the learning rate of old rats in a Greek cross-shaped nucleus. M.J.D. Misiendron* & M. Davis (SPONSOR: J. ROSEN) Yale Univ., Dept. of Psychiatry, Rhoicof Res. Fac., Conn. Mental Health Ctr., New Haven, CT. 06510.

The present results indicate that nimidinium may be an useful drug for the treatment of certain aspects of brain aging and support recent findings obtained from clinical studies with aged people treated with nimidinium.


We have studied the effects of drugs which affect brain polyamine levels on rat activity in a Greek cross-shaped maze, with two white and two black arms separated by a central gray compartment, comminucated with a central gray compartment. Total entries into peripheral compartments measure exploratory activity, while white entries are a measure of sensation-seeking behavior. Thirty day-old, male albino rats were used.

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0506.17


The selective melatonin receptor antagonist luzindole (LUZ) exerts antidiuretassic-like activity in the C5H/HeN mice behavioral despair test (Mogilniks & Dubocovich, Neurosci. Abs. 12, 1019, 1987). We therefore recently investigated the involvement of melatonin (MEL) in the behavioral despair test using LUZ. The time of immobility (sec) during testing was determined in the dark phase of a 14.10:1 h, L/D cycle in C5H/HeN mice which receive MEL and in C57BL/6 mice which do not receive the hormone. In controls, the duration of immobility was 55 + 5 (n=7) in C5H/HeN and 147 ± 8 (n=24) in C57BL/6. Desipramine (30 mg/kg, i.p.) significantly reduces the duration of immobility in C5H/HeN (39 ± 4, p<0.001, n=6) and in C57BL/6 (104 ± 18, p<0.001, n=8). LUZ (10 mg/kg, i.p) reduces the duration of immobility in C5H/HeN (17 ± 3, p<0.001, n=31) but not in C57BL/6 (170 ± 3, n=24). While MEL (30 mg/kg, i.p.) did not affect the time of immobility in C5H/HeN (52 ± 5, n=24) it reversed the anti-immobility effect of LUZ (3 ± 9, n=15). The lack of effect of LUZ in C57/BL6 mice and the antagonism of its effect by MEL in C5H/HeN mice suggest an involvement of endogenous MEL in the behavioral despair test. It is suggested that the antidiuretassic-like activity of LUZ occurs through a different mechanism than that of classical antidiuretics.

Supported by DK-38607 and Nelson Research.

0506.18


CP-55,940 is a nonclassical cannabinoid analgesic agent whose ability to inhibit adenylyl cyclase in a neuronal cell line has been reported (Howlett et al., Mol. Pharm. 33:297, 1988, chmp 8). CP-55,940 was used to characterize a receptor site in brain. Using a Ph2 prepara tion from rat cortex, a binding site was characterized which was saturable with either 1 µM CP-55,940 or delta-9-tetrahydrocannabinol. Cannabinol and cannabidiol, cannabionoid drugs lacking antidiuretic activity, did not displace CP-55,940. Scatchard analysis of saturation curves indicated a high affinity site having a Kd of 110 nM and a lower affinity site having a Kd of 1.4 µM. Kd values calculated from the association and dissociation rates were similar. Guanlylimidodiphosphate eliminated the binding to the high affinity site, and increased the number of low affinity sites, indicating that a characteristic interaction of the receptor with G-proteins is likely. Divalent cations increased binding, and Na and other monovalent cations decreased binding of CP-55,940. This behavior is characteristic of other receptors associated with inhibition of adenylyl cyclase.

(Supported by DAO3690, NS07254, and NS08686.)

0507.1

CALBINDIN D-28K PROTECTS AGAINST GLUTAMATE INDUCED NEUROTOXICITY: STUDIES IN TISSUE CULTURE. G. E. Bading and J. Kan* Dept. Physiology, University of British Columbia, Vancouver, B.C., Canada. VAT 14S.

When neuronal cultures of rat CA1 pyramidal cells are exposed briefly to high levels of glutamate a delayed, (24hr), and Ca++ dependent neuronal death occurs. Depending upon the glutamate concentration, however, some neurons live. We have now analyzed the survival and glutamate stimulated intracellular Ca++ concentrations using neurons loaded with Fura II (3) and in C57BL/6 (104 ± 18, p<0.001, n=8). LUZ (10 mg/kg, i.p.) reduces the duration of immobility in C5H/HeN (17 ± 3, p<0.001, n=31) but not in C57BL/6 (170 ± 3, n=24). While MEL (30 mg/kg, i.p.) did not affect the time of immobility in C5H/HeN (52 ± 5, n=24) it reversed the anti-immobility effect of LUZ (3 ± 9, n=15). The lack of effect of LUZ in C57/BL6 mice and the antagonism of its effect by MEL in C5H/HeN mice suggest an involvement of endogenous MEL in the behavioral despair test. It is suggested that the antidiuretassic-like activity of LUZ occurs through a different mechanism than that of classical antidiuretics.

Supported by DP-38607 and Nelson Research.

0507.2

PHOTOLYTIC EFFECT OF POLYLYSYL-PHTHALOCYANINE DERIVATIVES ON MURINE NEUROBLASTOMA CELLS. S. Korgoth, F. Sieber* and T. Kalinke University of WI, Madison WI 53706 and Med. Coll. WI, Milwaukee WI.

Poly-lysyl-phthalocyanine (PL) was identified as a potential serotonin-agonist by virtue of an increased uptake of 5-hydroxytryptamine (5HT) and an increased accumulation of serotonin in presynaptic nerve endings of the neuronal culture. However, after light exposure, the uptake of 5HT was reduced. The effect of light exposure of the neuronal cultures is characteristic of other receptors associated with inhibition of adenylate cyclase.

(Supported by D03690, NS07254, and NS08686.)

0507.3


To examine the mechanism of halothane toxicity that was characterized by the suppression of axonal and dendritic extension, actin distribution in fibroblasts in vitro, was studied by immunocytochemistry using the anti-biotin peroxidase-antibiotin technique. Rat 3T3 fibroblasts were cultured in the presence of 1% halothane in gas phase up to 4 hours. The growth rate of the cells was significantly slowed by halothane. Although PgFPP (the small subunit of actin) was not stained. Analysis of staining intensity of cells by an image analyzer clearly supported such qualitative observations. It appears that the effects of halothane on neuronal extension is a reflection of the microfilaments inability to function in the presence of halothane. Supported by the Koch of Dimes Birth Defects Foundation (grant no. 15-56).

0507.4


Intracellular Ca ion concentrations [Ca2+]i were measured in NG108-15 cells using the intracellular divalent cation indicator, 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (5F-BAPTA) and fluorine-19 Nuclear Magnetic Resonance Spectroscopy (NMR). This methodology provides for the simultaneous identification and measurement of [Ca2+]i and a variety of heavy metals, including Pb2+.

The NG108-15, a hybrid cell line of murine neuroblastoma and rat pheochromocytoma, is sensitive to chemically induced differentiation and co-culturing with fetal rat myoblasts. NG108-15 cells were grown on poly-D-lysine (PDL) coated glass coverslips in Dulbecco's Modified Eagle's Medium (GIBCO) supplemented with 10% foetal bovine serum. Cells were loaded with 5F-BAPTA and superfused at 2 ml/min with oxygenated medium during NMR observation. NMR measurements were performed on a Varian VXR 500 using a 10 mm broad band probe tuned to 470 MHz for fluorine. Using this methodology the average [Ca2+]i was measured to be 105 nM. Thus, NG108-15 cells will be used as a model to study the effects of Pb on the early events leading to synaptogenesis, specifically as it affects [Ca2+]i.

In order to better understand the pathogenesis of neurofliament (NF) and neurotrophic factor changes in aluminum (Al) neurotoxicity, we have begun to explore the effects of Al lactate on cell cultures. Rapidly dividing neuroblastoma x glioma hybridoma (N2A-B-1) cells, passages 24-28, were cultured for up to eight days in concentrations of Al lactate (0.5-6 mM). Cell growth, as determined by tritiated thymidine incorporation, was not reduced until Al in the media exceeded 2 mM. In the presence of 1-2 mM Al, the activity of choline acetyltransferase (ChAT) was increased (132% of control), whereas glutamate decarboxylase (GAD) activity was unchanged from control. Saturation kinetics showed an increased amount of ChAT in control cells. Al neurotoxicity is a complex phenomenon known to affect both synaptic neurotransmission and cytoskeletal proteins. The N2A-B-1 cell line is a useful in vitro model for further characterization of the effect of Al on the cholinergic system.


Primate adrenal medullary cells were exposed to 1 methyl-4 phenyl-1,2,3,6 tetrahydropyridine (MPTP) in an in vitro model. Cells were differentiated by prostaglandin Eı and dibutyryl cyclic AMP to induce a neuronal phenotype. Both differentiated and mitotic cells were exposed to MPTP (3.4-304 µM) for 24-72 hr.

Differentiated cells were less sensitive to or protected from MPTP toxicity as compared to mitotic cells. Toxic response was seen morphologically as increased cytoplasmic inclusions, loss of neurites, loss of intact cells and cell death. Measurements made after 24 hr showed the day 0 toxicity as a post-continued morphologic findings of protection with differentiation.

To further explore the benefits of differentiation, cells were treated with mixed gangliosides(GO/2900 mg/ml), a neurotropic factor in the CNS. GA have been shown to aid in neuronal recovery in vivo from MPTP toxicity(Hadjiconstantinou M. et al., Neuropharm. 25:1075, 1986). GA induced neurite formation in both mitotic and previously differentiated cells. 24 hr pre-treatments of GA with MPTP reduced the loss of (3H)-leucine incorporation during protein synthesis and prevented the loss in total protein induced by MPTP in mitotic and differentiated cells. GA aided in the recovery of both mitotic and differentiated cells following removal from MPTP exposure. Recovery included cell proliferation and the extension of neurites.

This in vitro model of neurotoxicity provides a system in which agents can be tested for their ability to protect against a neurotoxin.

Supported in part by NIHES Pilot Grant from ES01247.


The effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were studied on a human neuroendocrine cell line in vitro before and after differentiation. LA-N-1 cells, which are neural crest derivatives, are adrenergic and differentiate morphologically and biochemically when treated with 10-5 M retinoic acid (RA), a vitamin A antagonist.

Miotic cultures exhibited a dose dependent decrease in cell number after treatment with 1, 10 or 50 µg/ml MPTP (1%, 4% and 23% respectively). Cultures treated with 1 µg/ml had similar growth curves to control cultures. Cultures treated with 10 µg/ml showed a 35% decrease in cell number by day 2 of treatment, while the decrease remained constant throughout the 5 day test period. Cultures treated with 50 µg/ml exhibited an 85% decrease in cell number by day 2 and continued to decrease to 95% by day 5. Cell numbers in differentiated cultures were reduced both the fraction of neurons growing neurites (IC50 = 226 µM), but did not reduce the mean neurite length per neurite-growing cell. Triethyl lead reduced both the fraction of neurons growing neurites (IC50 = 0.25 µM) and the mean neurite length (IC50 = 0.42 µg/ml).

Supported by grants from NIH (ES03158) and EPA (R-813228) to G. Audesirk.
507.11 EFFECT OF LEAD AND MERCURY ON LIPID METABOLISM OF SCIATIC NERVES IN CULTURE. R.J. Hoffman and A. Halter. Dept. of Pharmaceutical and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Reaggregate tissue cultures composed of neurons from the mesencephalic tegmentum and corpus striatum of 14 day old embryonic mice were treated with methamphetamine (mam; 10-4 to 10-5 M) at 15 days in culture for 7 days. This caused parallel decreases in dopamine (DA) cell numbers (visualized by histofluorescence) and reductions in serotonin (5HT) levels. Accumulation of exogenous DA expressed per visualized DA neuron and endogenous 5HT levels were not altered by this treatment. Time-course studies showed that the levels of DA are decreased by 38 and 68% of control after 1 day of treatment with 10-5 M or 10-6 M meth at 15 days in culture and remained depressed after 4 and 7 days of treatment. 10-6 M meth decreased DA levels to 63% of control but only after 7 days of treatment. Serotonin levels decreased gradually between 1 and 7 days of treatment with 10-6 M and 10-7 M meth but were not decreased until after 4 days of treatment with 10-6 M meth. Thus, it appears that the levels of DA decreased more rapidly than those of 5HT at any given concentration of meth. The potential recovery from effects of meth was assessed by treating reaggregates with 10-6 M or 10-7 M meth for 2 days, discontinuing exposure to meth and allowing the reaggregates to continue in culture for an additional 5 days. Dopamine and 5HT levels were reduced after 2 days of treatment with meth. However, 5 days after removal of meth, DA levels had recovered while 5HT levels were still decreased. These experiments indicate that the decreases in DA levels can be reversed after removal of meth while the reduction in 5HT levels appear to be maintained. Supported by MH42134 and NIDA contract #2271-BX114.

507.12 NEUROTOXIC EFFECTS OF METHAMPHETAMINE ON DOPAMINE AND SEROTONIN NEURONS IN REAGGREGATED TISSUE CULTURE. J.H. Koob, R. Dinca, C. Hoffmann, and A. Halter. Dept. of Pharmaceutical and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Reaggregate tissue cultures composed of neurons from the mesencephalic tegmentum and corpus striatum of 14 day old embryonic mice were treated with methamphetamine (mam; 10-4 to 10-5 M) at 15 days in culture for 7 days. This caused parallel decreases in dopamine (DA) cell numbers (visualized by histofluorescence) and reductions in serotonin (5HT) levels. Accumulation of exogenous DA expressed per visualized DA neuron and endogenous 5HT levels were not altered by this treatment. Time-course studies showed that the levels of DA are decreased by 38 and 68% of control after 1 day of treatment with 10-5 M or 10-6 M meth at 15 days in culture and remained depressed after 4 and 7 days of treatment. 10-6 M meth decreased DA levels to 63% of control but only after 7 days of treatment. Serotonin levels decreased gradually between 1 and 7 days of treatment with 10-6 M and 10-7 M meth but were not decreased until after 4 days of treatment with 10-6 M meth. Thus, it appears that the levels of DA decreased more rapidly than those of 5HT at any given concentration of meth. The potential recovery from effects of meth was assessed by treating reaggregates with 10-6 M or 10-7 M meth for 2 days, discontinuing exposure to meth and allowing the reaggregates to continue in culture for an additional 5 days. Dopamine and 5HT levels were reduced after 2 days of treatment with meth. However, 5 days after removal of meth, DA levels had recovered while 5HT levels were still decreased. These experiments indicate that the decreases in DA levels can be reversed after removal of meth while the reduction in 5HT levels appear to be maintained. Supported by MH42134 and NIDA contract #2271-BX114.


In a second set of experiments, Schwann cells isolated from sciatic nerves of 4 day old mice were cultured for 15 days, then incubated 18 hours at 37°C with various concentrations of lead or mercury in the presence of one of the same precursors. Lipids were then extracted, separated, and level of the radioactivity was measured. Lead did not affect lipid metabolism of endoneurium and Schwan cells in culture, consistent with the hypothesis that Schwann cells are resistant to low concentrations of lead. 10-5M HgCl2 caused an increase in the incorporation of acetate into phospholipids of endoneurium and modified the pattern of acetate incorporation into lipids of both endoneurium and Schwann cells in culture. With acetate as a precursor, 10-5M HgCl2 modified only the pattern of its incorporation into lipids of endoneurium. These results suggest that the effect of mercury on lipid metabolism in Schwann cells depends on the state of functional differentiation of these cells.

507.14 Endogenous Mechanisms of Protection Against Catecholamine Toxicity in Rat Cerebral Cortex in Dissociated Cell Culture. S. Vasquez and P.A. Rosenberg (SPON: S. Fischer). Dept. of Neurology, Children’s Hospital and Harvard Medical School, Boston, MA 02115.

We have previously demonstrated the toxicity of noradrenaline (NE) and other catecholamines to both cortical neurons and glia in dissociated cell culture (J. Neurosci. 8: 1, 1988). The effect occurs at or below 10-5M NE, does not appear to be mediated by adrenergic receptors, and is blocked by catalase. Three types of cultures were used for experiments described: all contained approximately 40% of neuronal and 60% of glial cell types, used at 4-8 weeks in vitro; 2) ‘neuronal-culture’ cultures comprised of 70-90% neurons, used at 2-4 weeks in vitro. Cultures were exposed to NE for 24 hours in vitro. Two types of cultures were used: neuronal cultures, vulnerable to toxicity of 25 µM NE was found to be highly dependent upon culture density. At 250 µM NE this effect of culture density was not observed.

Further work will attempt to characterize the mechanisms of protection involved as well as their localization, developmental expression, and regulation. This work was supported by a Robert Morison Fellowship from the Grass Foundation, NS 00993, and CHMR Core HD 06276.

508.1 THE ROLE OF NOC2 IN GM1-MEDIATED NEUROGENESIS. H.T. Safarstein and P.J. Sileno (SPON: R. Deymanjan). Department of Anatomy and Neurobiology, School of Medicine, Univ. of Louisville, Louisville, KY 40292.

Exposure of the murine neuroblastoma cell line Neuro-2a to the ganglioside GM1 has been shown to facilitate neurogenesis. The neurotrophic action of GM1 on Neuro-2a cells is blocked by catalase, suggesting that GM1 activation of microtubule-binding proteins is involved. In this study, the role of the neural cell adhesion molecule (NCAM) in GM1-mediated neurogenesis was examined by modifying the availability of NCAM in dissociated embryonic rat cortical cultures. The goal of this study was to examine the expression of NCAM in GM1-treated cultures by comparing the distribution of NCAM in GM1-treated cultures with those in control cultures and non-treated cultures. The results of this study indicate that the distribution of NCAM in GM1-treated cultures is different from that in control cultures and non-treated cultures. Moreover, the expression of NCAM in GM1-treated cultures is different from that in control cultures and non-treated cultures. Therefore, the expression of NCAM in GM1-treated cultures is different from that in control cultures and non-treated cultures. This difference is not due to changes in the expression of NCAM but rather to changes in the distribution of NCAM. The results of this study suggest that GM1-mediated neurogenesis may be mediated by changes in the distribution of NCAM rather than by changes in the expression of NCAM.


Thy-1, a major neuronal surface glycoprotein, is the simplest member of the immunoglobulin superfamily. Its expression on neuronal axons is thought to be involved in mediating, as yet undefined, cellular interactions in nervous tissue. However, neuronal-axonal cultures were exposed to 25 µM NE. Further work will attempt to characterize the mechanisms of protection involved as well as their localization, developmental expression, and regulation. This work was supported by a Robert Morison Fellowship from the Grass Foundation, NS 00993, and CHMR Core HD 06276.

MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES III
The protein 1B26 was originally identified by analysis of rat brain-specific cDNA clones and has been shown to be identical to myelin-associated glycoprotein (MAG). A single 1B26/MAG gene has been mapped, (D'Eustachio, P., et al, J. Neurochem., 50:589, 1988; C. Blatt, personal communication), near the basal lamina of chromosome 7 of the mouse. Quivering is a recessive mutation in mouse that is characterized by a progressive instability of gait.

We have previously observed 1B26/MAG mRNA expressed in different mouse strains using RNase protection experiments. These probably correspond to allelic differences in the 1B26/MAG gene: one form was found in most mouse strains tested while the second was detected only in DBA/2 and in quivering mice. Because the quivering mutation (qv-q) used in these studies arose in a DBA/2 strain mouse, the expression of the DBA/2 allele of 1B26/MAG in quivering mice, but not in the background strain (C3HeB6) for the mutant, suggests that the quivering and 1B26/MAG genes are very closely linked, if not identical.

The mapping data combined with the results of our RNase protection experiments have led us to investigate these allelic differences more thoroughly. We are using nuclear acid sequence analysis to compare 1B26/MAG cDNAs from DBA/2, C3HeB and quivering mice. Preliminary data has revealed differences in the sequences of the C-terminal end, which is thought to be the limits of structural variability allowable in the 1B26/MAG gene product. (Supported by a Fellowship from the National Multiple Sclerosis Society to L.H.F. and grant NS 20728 from NIH).

The antigen, recognized by this antibody, has been affinity purified and appears as band of 75 kDa on a Western blot. The antigen is highly expressed in developing zebrafish, reaching maximal expression at 3 days after fertilization. Expression then decreases rapidly and by 14 days in extremely low. This temporal pattern of expression correlates with the time these neurons are extending axons and making synapses.

The Ze-12 antibody labels sensory neurons quite specifically in early development but later recognitions nearly all neurons. Affinity purification yields a major band at 65 kDa on a Western blot, although an extremely heterogeneous band is measured in adult brain extracts with a range of molecular weights from 65 to more than 200 kDa. Supported by NIH grant HD 22486.

**508.4**

**1B26/MAG EXPRESSION IN NEURONS C. LAI, E.L.F. BATTENBERG, R.J. MILNER, AND J. BLOOM. Research Institute of Scripps Clinic, La Jolla, CA 92037.

The rat protein 1B26 was isolated as a brain-specific gene product and has been shown to be identical to the myelin-associated glycoprotein (MAG). This protein exists in two forms, a longer form whose expression is maximal at approximately postnatal day 25, and a shorter form whose expression peaks at approximately postpartum day 21. We have previously observed 1B26/MAG immunoreactivity in a subset of neurons in both adult and young rats (Bloom, F.E. et al, J. Neurosci., 5:1781, 1985 and Lenoir, D. et al, J. Neurosci., 6:522, 1986). Here we extend these analyses using antibodies generated against MAG to examine young (postnatal day 21) and adult (>3 months) rats as well as 75 day old mutant quivering (qv-q) and normal background strain (B6C3Fe) mice. One rabbit polyclonal and two mouse monoclonal antibodies against MAG (gifts of R.H. Quarles, NINCDS) each show a pattern of immunoreactivity similar to that seen with the 1B26 anti-peptide antibodies used in the original surveys. All of these antisera recognize both oligodendrocytes and neurons and reveal a near coincident pattern of expression. In situ hybridization studies were conducted utilizing oligonucleotide probes that distinguish between the mRNAs encoding the two protein forms. Hybridization is observed to mRNA contained within oligodendrocytes and neurons in a pattern consistent with the immunohistochemical results. 1B26/MAG is believed to function as a cell adhesion molecule in oligodendrocytes; whether neuronal 1B26 expression is present in vivo remains to be established. Supported by NIH grant NS 20728.
508.10 IMMUNOREACTIVITY TO ANTI-SYNAPTIC VESICLE PROTEIN IN THE RETINA AND PARIETAL EYE OF A LIZARD. G. A. Engstrom and M. A. Rinehart*. Institute for Sensory Research, Syracuse University, Syracuse, New York 13244.

The parietal eye and retina are two distinct vertebrate photoreceptive organs which display some structural and functional commonalities. Retinal and parietal eye tissues were examined for immunoreactivity to anti-synaptic vesicle protein antibodies. The antibodies label a 36 kDa protein (SVP-36) from the synaptic fraction of guinea pig cerebellum and they have been seen to label synaptically associated structures in several vertebrate classes.

In the retina the immunoreactivity was heavy in the inner plexiform layer (IPL) with distinctive banding in sublayers 2, 4, and 5. Labeling was also found surrounding most of the cells near the IPL-inner nuclear layer boundary, probably amacrine cells. Very light labeling was found in the outer plexiform layer (OPL) and was mostly restricted to a thin layer where the OPL and the horizontal cells meet. Some immunoreactivity was localized in patches in the optic nerve fiber layer. In the parietal eye the immunoreactivity was similar in density to the retinal OPL labeling though not layered in any discernable pattern.

The immunoreactivity to anti-SVP-36 is to be expected in the IPL of the retina. Electron microscopy showed a high density of synaptic junctions there. However, the greatest density of synaptic vesicles in both the retina and parietal eye was seen in the photoreceptor synaptic terminals, where anti-SVP-36 immunoreactivity is sparse. Evidently synaptic vesicles from photoreceptors differ in composition from those in neurons of the IPL.

We thank Dr. K. Obata for his generous gift of antibodies.

Supported by NIH grant EY-03539.


Schwann cells isolated from rat sciatic nerve express high levels of NGF receptors when maintained in primary tissue culture. Experiments were carried out to examine the ability of neurons or neuronal membranes to regulate Schwann cell NGF receptor number when co-cultured with Schwann cells. Mouse superior cervical ganglion (SCG) neurons were co-cultured with rat Schwann cells for 1 wk. Schwann cells were assayed for NGF receptors using a 2-site immunoassay specific for rat NGF receptors. Mouse SCG neurons induced Schwann cell proliferation followed by down-regulation of Schwann cell NGF receptor binding. The degree of receptor down-regulation was dependent on the number of neurons co-cultured with Schwann cells. Crude membranes prepared from postnatal day 7 (P7) mouse sciatic nerve or spinal cord also caused proliferation and down-regulation of Schwann cell NGF binding after 3 days of co-culture. Membranes from P7 cerebellum, cerebral cortex, or liver had no receptor regulating activity. Membranes from P0, P14 and adult mouse spinal cord were also effective in regulating Schwann cell NGF receptor number. Preliminary experiments showed that receptor regulating activity was solubilized from spinal cord membranes with Triton-X-100 and that upon boiling the extracts activity was lost. These results show that an NGF receptor regulating activity is found in axon-rich preparations from the both the central and peripheral nervous systems.


Multiple variants of gap junction proteins called Connexins (Cx) have been demonstrated in several tissues. The variants include Cx32 (the subscript referring to molecular weight in kD) first detected in liver, and Cx43 first detected in heart. Previous studies from our laboratories as well as others have demonstrated the presence of Cx32 and Cx26 in adult rat brain, specifically in cerebellum. The present study was carried out to investigate the presence of Cx32 in adult rat brain and in cells cultured from fetal rat brain. Neuronal and astroglIAL cultures prepared from 18-day fetal telencephalon and newborn cerebral cortex respectively were maintained in a serum-free defined medium. A polyclonal anti-peptide antibody to the first 20 N-terminal amino acids of Cx32 was affinity purified using the synthetic peptide. SDS PAGE and western blotting of homogenates indicated the presence of a 38 and 40 kD proteins in brain homogenates compared to 38 and 43 kD proteins in heart homogenates. Although both 38 and 40 kD bands were observed in astroglIAL and neuronal cultures, the latter was markedly more prominent in glial cultures. Degradation products of identical MW were seen in both heart and brain homogenates. These results suggest that gap junctions may play a role in the development and regulation of CNS function. Supported by NIH grants HL37109-03 (BHN) and ROI NS24629-02 (REF).
HIGH AFFINITY CHOLINE UPTAKE OF HIPPOCAMPAL SYNAPTOSOMES IN RESPONSE TO ENDURANCE TRAINING IN YOUNG AND OLD RATS
Aging has been shown to decrease hippocampal cholinergic function. Endurance exercise has been shown to modify characteristics of brain neurons and their receptors. Due to the dramatic increase in hippocampal in
invasion and control of voluntary movement as well as memory, it was of interest to determine whether endurance exercise would alter acetylcholine metabolism of the hippocampus in young and old rats. High affinity cholinergic uptake, the
limiting step in acetylcholine synthesis, was determined in synaptosomes of the hippocampus of endurance trained rats and their age-matched sedentary controls. Male F344 rats were run on a treadmill for 509.5 m/min. Young rats, originally at 6 months of age, and old rats, originally at 19 months of age were killed by decapitation at 12 and 25 months of age, respectively, and the synaptosomes of the hippocampal isolated. The high affinity cholinergic function was therefore determined by incubating the synaptosomes with 0.75 µM (1,3 histidine) choline chloride. There was a significant increase in the uptake of choline accumulating and sodium-free medium. Comparison of synaptosomes of untrained, young or old rats showed a 35% (p<0.05) increase in high affinity choline uptake which is consistent with previous reports of an age-related reduction of cholinergic function. The synaptosomes of young trained rats demonstrated a 28% (p<0.02) reduction (13.5 pmol/mg vs. 10.8 pmol/mg) in high affinity choline uptake. This reduction indicates alterations in presynaptic cholinergic function which may influence cholinergic synaptic transmission and/or acetylcholine turnover. Old trained rats showed no significant difference from their age-matched controls indicating a loss of synaptic adaptability in aged animals.

AGE-RELATED CHANGES IN &-ADRENERGIC RECEPTOR DENSITY IN RAT BRAIN: AUTORADIOGRAPHIC ANALYSIS USING QUANTITATIVE AUTORADIOGRAPHY
D.M. BURNETT and N.R. Zahniser Dept. Pharmacology, Univ. Colorado HSD. Sol. CLR., Denver, CO 80262
It is well documented that the electrophysiological responsiveness of the CNS to noradrenergic stimulation is diminished with aging. To determine whether receptor changes accompany this diminished responsiveness, we have studied the effects of aging on the density of &-adrenergic receptors (ARs) in circumbrochinal areas of the rat brain using computer-assisted quantitative autoradiography. Sagittal sections of Fischer 344 rat brain were prepared from three aged groups: 4-5, 16-18 and 24-28 months. Saturation curves were generated using 1-200 µM (-) H[125]I-HI-2554 (IE), an &-selective antagonist. Nonspecific binding data defined 60% to 70% of the total binding in discrete brain areas was normalized by measuring corresponding protein levels with a densitometric image analysis. Scatchard analysis revealed significant age-related decreases in the density of &-ARs in the thalamus and olfactory tubercle, but not in cortex, striatum, hippocampus, cerebellum or brain stem. In the thalamus basis (+)-50 fmol/mg protein at 4 months of age vs. 771 ± 83 fmol/mg protein at 24-28 months. In the olfactory tubercle, corresponding values were 425 ± 58 vs 294 ± 45. No affinity changes were found. Scatchard analysis and binding data suggest that significant decreases in the number of &-ARs are also evident by 16-18 months of age. These findings were found that aging, decreases in the number of central &-ARs are not ubiquitous but are confined to specific areas. Supported by USPHS AG-04418 and the PMA Foundation.

EXTREME SHIFTS IN CHOLINE UPTAKE AND ACETYLCHOLINE SYNTHESIS IN RESPONSE TO ENDURANCE TRAINING IN YOUNG AND OLD RATS
Aging has been shown to decrease hippocampal cholinergic function. Endurance exercise has been shown to modify characteristics of brain neurons and their receptors. Due to the dramatic increase in hippocampal in
invasion and control of voluntary movement as well as memory, it was of interest to determine whether endurance exercise would alter acetylcholine metabolism of the hippocampus in young and old rats. High affinity cholinergic uptake, the
limiting step in acetylcholine synthesis, was determined in synaptosomes of the hippocampus of endurance trained rats and their age-matched sedentary controls. Male F344 rats were run on a treadmill for 509.5 m/min. Young rats, originally at 6 months of age, and old rats, originally at 19 months of age were killed by decapitation at 12 and 25 months of age, respectively, and the synaptosomes of the hippocampal isolated. The high affinity cholinergic function was therefore determined by incubating the synaptosomes with 0.75 µM (1,3 histidine) choline chloride. There was a significant increase in the uptake of choline accumulating and sodium-free medium. Comparison of synaptosomes of untrained, young or old rats showed a 35% (p<0.05) increase in high affinity choline uptake which is consistent with previous reports of an age-related reduction of cholinergic function. The synaptosomes of young trained rats demonstrated a 28% (p<0.02) reduction (13.5 pmol/mg vs. 10.8 pmol/mg) in high affinity choline uptake. This reduction indicates alterations in presynaptic cholinergic function which may influence cholinergic synaptic transmission and/or acetylcholine turnover. Old trained rats showed no significant difference from their age-matched controls indicating a loss of synaptic adaptability in aged animals.

CYTOSOLIC CALCIUM CONCENTRATIONS IN CORTICAL SYNAPTOSOMES OF AGING RATS. L. Giovannielli and G. Pennesi. Dept. of Pharmacology, Florence University, 50134 Florence, Italy. Cytoxic calcium concentrations were measured in purified synaptosomes from the cerebral cortex of 3, 16 and 24 month old male Charles River Wistar rats, by the Quin-2 technique. Electron microscopy examination of the synaptosomes revealed an 13% concentration in mitochondria. Synaptosomes were incubated at 37 °C in oxygenated medium, pH 7.4, and loaded with 20-30 µM Quin-2AM for 15 min. After dilution, centrifugation and resuspension of the samples, readings were made in a Perkin Elmer spectrophotometer. Calcium concentration at rest (124±6 nM) nor the increase after potassium (50 mM) depolarization was modified by age. The calcium load following depolarization was cleared in about 13 min in 3 month old rats. The rate of clearance was significantly slower in 24 month old rats. The addition of verapamil (60 µM) or nisoldipine (10 µM) after depolarization restored calcium concentration to resting level in aged at the same rate as in young rats. An increase in calcium influx mediated through L channels may therefore be responsible for the slower clearance of calcium load in aged rats. Work supported by a CNR grant.

AGING AND MUSCARINIC RECEPTOR SUBTYPES: AUTORADIOGRAPHIC ANALYSIS IN RAT AND HUMAN BRAIN. A. Blegen, E. Farley*, M. Hanan, and M. Segal. Weizmann Inst. Sci., Rehovot, IS.
As a step towards the characterization of age effects on the cholinergic system, we compared the distribution of muscarinic M1 and M2 receptors in brains from young and old subjects, using quantitative in vitro receptor autoradiography. Twenty four and fifty brain sections from young (age range 17 to 81 years) were collected from the autopsy material of the Neuropathology Research Institute, New York University. Seven young (4 months) and 6 old (28-31 months) rats were used. Rats were run on a 10-fold higher concentrations of 20-30 µM calcium or 2000-3000 µM pirenzepine respectively. Specific binding was assessed in the presence of 10 µM atropine. The autoradiograms were analyzed by an IBM PC based computerized imaging system. A pattern of anatomically selective, age-related decreases in M1 and M2 receptors over time in human brain was observed in both human and rats. M1 receptors were significantly decreased (10-30%) in cortex striatum and several subregions of the hippocampus. M2 receptors in cortex and striatum were decreased, while striatum which maintained levels. A large decrease (more than 50%) in M2 receptors was observed in the substantia innominate and other ventral forebrain cholinergic nuclei. The location and size of the age-related changes were similar in human and rat.
509.7 Age-Related Electrophysiological Changes in Cerebellar Nodornergic Receptors. The role of age in the regulation of neurotransmitter receptor action was examined in the cerebellum. Previous examinations have shown decreases in noradrenergic transmission in the central nervous system of aged laboratory animals. In particular, sensitivity to locally-applied a-phenylephrine has been observed when recording miniature end-plate potentials in cultures of cerebellar Purkinje cell. It was the purpose of this study to determine which, if any, of these receptors are altered as a function of age. The ability of selective noradrenergic agonists to inhibit the spontaneous activity of Purkinje neurons was compared in young (3-mo.) and old (18- and 26-mo.) F344 rats. Drugs were applied to Purkinje cell by pressure micro-injection from multi-barreled micropipettes and a paired-pipette paradigm was used to control the potency of each pipette in young and aged rats. Purkinje cell from young rats were significantly more sensitive to locally-applied isoprenaline, a beta- adrenergic agonist, than Purkinje cell of both the older age groups. Sensitivity to the alpha agonist phenylephrine and the alpha agonist clonidine was not observed in the aged rats. These results suggest that post-synaptic sensitivity to beta-adrenergic agonists decreases with senescence, whereas postsynaptic sensitivity to alpha and alpha agonists does not change.

509.8 NEUROMOTOR COORDINATION MECHANISMS AND AGING. N.C. Rich, \(\text{Ph.D.,} \)and E.S. McNaughton, Brainwave Systems Corp., Boulder, CO 80305.

The purpose of this investigation was to examine neuromotor coordination mechanisms in young and old males during ballistic forearm flexion and extension tasks. The subject sample included 16 males in each of the following groups: 1) 30-40-year-old, 2) 50-60-year-old, and 3) 60-70-year-old. The IEMG patterns of the biceps (agonist) and triceps brachii (antagonist) muscles were recorded during unresisted and resisted trials. Sixteen temporal and ten quantitative parameters were computed to evaluate between group differences. The parameters which exhibited significant differences between groups were: (1) agonist first burst silent period, (2) antagonist burst duration, (4) agonist burst IEMG slope, (5) antagonist second burst time to peak activity, (6) time to maximum acceleration, (7) agonist first burst and antagonist second burst peak amplitude, and (8) acceleration as a percentage of movement time. For forearm flexion strength groups 2 and 3 were significantly stronger than group 1. Further, for forearm extension strength groups 2 and 3 were 89.12% and 83.90% as strong as group 1, respectively. The data indicate that while loss of strength is indeed a true concomitant of the aging process, an individual's muscle patterns remain relatively stable throughout these decades.

509.9 DIFFERENTIAL INFLUENCE OF THE MEDIAL SEPTUM ON CA1 "PLACE" CELLS AS A FUNCTION OF AGE. S. L. Markowska, A. L. Markowska, C. A. Barnes, and B. L. McNaughton, Dept. Psychology, University of Colorado, Boulder, Colorado 80309.

To examine the effects of aging on septal modulation of the behavioral correlates of different hippocampal cell types, 4 young (11 mo) and 4 old (25 mo) Fischer 344 rats were trained to perform a working memory task on an 8-arm radial maze. Cereal hippocampal neurons were recorded using stereotopes (McNaughton et al., \textit{J. Neurosci.} 1983), mounted on moveable microvires. A guide cannula was also implanted to permit local application of drugs to the lateral septal nuclei. When hippocampal units were encountered, the unit-behavior correlate was characterized during the first and during the subsequent sessions. The septum was then reversibly inactivated via tetracaine injection (0.5 µl). Post-injection behavioral performance and unit activity were monitored for an additional 10 trials.

Following septal inactivation, place-specific firing was maintained in 91% of the CA1 "place" cells (N=22) from young rats, in spite of severely impaired performance on the maze and a dramatic reduction in activity of 64% of dentate gyrus cells (N=50), which include hit "place" cells, basket and granule cells (also reported by Mizumori et al., \textit{J. Neurosci.} 1987). In contrast, only 25% of CA1 "place fields" (N=24) from old rats were maintained during the period of behavioral impairment. Similar to young rats, 75% of dentate cells from old rats (N=57) showed reduced firing after tetracaine injection. These findings suggest at least the following two possibilities: 1) the inputs to the "place" cells from within the hippocampus are more highly distributed in young rats than in old rats, and/or 2) direct inputs from entorhinal cortex to CA1 maintain place fields in young, but not old rats.

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509.10 AGE-RELATED DECREASE IN PERIPHERAL PATH EVOKED SPIKE ACTIVATION IN RAT FACIA DENTATA. C.A. Barnes, C.A. Barnes, B.L. McNaughton, and G. Spangler, Department of Psychology, University of Colorado, Boulder, Colorado.

The purpose of this experiment was to examine the effects of aging on septal modulation of the behavioral correlates of different hippocampal cell types. In particular, a hippocampal neuronal population was examined which follows pp stimulation. Sensitivity of hippocampal neurons to exogenously applied glutamate. A total of 354 FD, 307 CA1, and 298 CA3 cells were studied from 30 animals divided by their sum. Glutamate application produced a substantial regression on age of either the spontaneous background activity or the relative sensitivity of hippocampal neurons to exogenously applied glutamate.
AGING ELIMINATES THE DIURNAL RHYTHM AND DEPRESSES THE DENSITY OF SELECTED BRAIN AREAS.

N.G. Welland and P.M. Wise. Dept. of Physiology, Univ. of Maryland, School of Medicine, Baltimore, MD 21201.

The densities of α2-adrenergic receptors exhibit a diurnal rhythm in selected hypothalamic nuclei of overactinized (OXV) young rats. In intact rats at 1000 h α2 receptors decreased with age depending on the brain region and/or reproductive status. We wished to determine whether the age-related changes in α2 receptor density reflect a change in the diurnal rhythm and/or occur independently of reproductive status. Using autoradiographic procedures, we measured the density of α2 receptors in young, middle-aged, and old OXV rats at various times of day. In the medial preoptic nucleus, the diurnal rhythm of the density of α2 receptors was abolished in middle-aged rats, and receptor densities were decreased in old rats. In suprachiasmatic nucleus and ventral medial nucleus, α2 receptor densities were suppressed in middle-aged rats, and a further decrease occurred in old rats. In median eminence α2 receptor densities declined progressively and were significantly decreased in old rats. No age or time-associated changes in α2 receptor densities occurred in lateral septum. The data demonstrate that during middle-age there is an initial loss of the diurnal rhythm in α2 receptor densities followed by a progressive decrease in α2 receptor concentrations in older animals. AG-0537, AG-0224.


Intracerebroventricular (ICV) 1% ethanol was microinjected into the lateral ventricle of both young (<6 mo) and old (>15 mo) female rats. After 30 min, acute effects of ethanol were studied with extracellular recording in vitro. Both young and old rats exhibited a decrease in membrane conductance, the Ca-dependent, K-mediated afterhyperpolarization (AHP) and decreasing electrical excitability. There were no significant differences between young and old rats in any parameter. Ethanol decreased membrane conductance, AHP duration and action potential (AP) duration. EPSP amplitude and duration were reduced in young rats but not in old rats. AP duration increased in young rats but not in old rats. No significant changes were observed in hippocampus indicating an area selectivity of N channel impairment. On the other hand the observed changes in cortex may participate to the impairment of calcium uptake and neurotransmitter release observed in this brain area in aged rats.

ELECTROPHYSIOLOGICAL STUDY OF THE SUBSTANTIUM NIGRA PARS COMPACTA NEURONS IN YOUNG AND AGED WISTAR RATS. PRELIMINARY RESULTS. M.A. Lavín and R. Drucker-Colín. Instituto de Fisiología Celular, UNAM, México.

The substantia nigra pars compacta (SNC) cells are involved in motor control. There is evidence indicating that some characteristics of SNC cells are altered in aged rats (McGeer, E. and McGeer, L. Ergot compound and Brain function. Ed. by Goldstein, 1980).

Male Wistar rats young (3 months old) and aged (20 month old) were anesthetized with halothane, tracheostomized and fixed to a stereotaxic apparatus. Extracellular activity was recorded using single glass capillaries filled with 2X pontamine blue in 0.3 M Na acetate (pH=16.6). Tip positions were marked by ejecting the dye at the end of the experiment.

15 Cells was recorded in young rats with a frequency rate of 40.5 ± 23.4 spikes/10 sec (X ± S.D.) and 7 cells in aged rats with a frequency rate of 31.2 ± 11.6 spikes/10 sec. Although there is no difference in the average frequency rate, there is a difference in the pattern of discharge. In aged rats almost all cells (71%) have a tendency to discharge in bursts of 3 spikes, sometimes altered with a single spike. In young rats, 73% of the cells have a tendency to discharge in a single spike. This analysis and more recording of SNC cells are in progress.


Although the hippocampus is a major target structure for corticosterone (CORT), little is known about its neuronal effects (cf. McEwen, 1982). In addition to its presumed normal actions, CORT also appears to involve in motor control. There is evidence indicating that some characteristics of SNC cells are altered in aged rats (McGeer, E. and McGeer, L. Ergot compound and Brain function. Ed. by Goldstein, 1980).

In the present studies, therefore, we examined a defined Ca-dependent membrane conductance, the Ca-dependent K-mediated afterhyperpolarization (AHP), in young (4-7 mo) and aged (24-27 mo) adrenalectomized (ADX) and intact rats. In intracellular recordings from CA1 cells of the AHP... was abolished by AHP duration. The AHP could be lengthened in ADX rats treated with injections of CORT prior to study.

These results suggest that an Ca-dependent membrane conductance is partially Ca-dependent and that the impact of CORT on this conductance increases dramatically with aging. Because excessive Ca influx has been implicated in cell death, these findings raise the possibility that increased Ca influx may be a factor in accelerated neuronal deterioration with aging. (AG04542).
DIFFERENTIATION AND DEVELOPMENT IX FRIDAY AM


Previous work in our laboratory has suggested that there are two populations of Merrick cells associated with a follicular sinus complex developing sequentially. To test this, rat pups of different developmental stages were obtained from timed pregnancies. Trigeminal axons innervate the superficial demins in the myeleral region on GD1.0. GD1.5. By electron microscopy, Merrick cells were first seen associated with the vertical row sinus halts G and H on GD16.5, and were present in the neck region of the vibrissae. On GD17.5, this population had separated from the neck region and were present in the outer root sheath. All of these axons were contacted by a dense plexus of afferent fibers extending from the perifollicular region. These results support the hypothesis that axons of Merrick cells develop sequentially along a temporal gradient within a single cell type, with the innervation pattern of each step being specific to the embryonic period.

510.2  NEURON-RECEPTOR CELL INTERACTION DURING DEVELOPMENT OF THE INNER EAR: A HETEROCHRONIC GANGLION STUDY. T. R. Van De Water* and USPHS Grant #NS19462.

The development of afferent projections from the inner ear to the central nervous system is an important aspect of neuroanatomical development. In order to study this process, a ganglion study was performed in which ganglia of different developmental stages were injected with horseradish peroxidase (HRP) and Fluorogold (FG). Under anesthesia, five animals of each age were injected with HRP (2-3 ul) in one SMG and FG(1X, 2-3 ul) in the other SMG. Following the injection, the rats were sacrificed and processed for the HRP reaction. The development of afferent projections from the inner ear to the central nervous system was observed to be developmentally regulated. It was found that the projections from the inner ear to the central nervous system developed more rapidly in the young rats than in the older rats. This suggests that the development of afferent projections from the inner ear to the central nervous system is influenced by the developmental stage of the animal. The results of this study support the hypothesis that the development of afferent projections from the inner ear to the central nervous system is developmentally regulated.


The development of the mediodorsal nucleus of the human thalamus is a complex process that occurs during ontogenesis. Activity begins with the formation of afferent fibers that connect the mediodorsal nucleus with the neocortex. The afferent fibers are then followed by the development of efferent fibers that project to the neocortex. The efferent fibers are then followed by the development of inter-neuronal connections that are essential for the proper functioning of the thalamus. The Golgi study of the mediodorsal nucleus was performed in 10 human fetuses ranging in age from 12 gestational weeks to 36 gestational weeks. The results of the study suggest that the development of the mediodorsal nucleus is a complex process that occurs during ontogenesis. The study also supports the hypothesis that the development of the mediodorsal nucleus is influenced by the developmental stage of the animal.

In the mature ION, the neuronal perikarya are arranged in a convoluted manner. The dendrites of these neurons are directed perpendicularly into the neuropil. By embryonic day 10 (E10), virtually all the dendritic trees are highly polarized, but, unlike in the mature ION, most are directed ventralwards or ventromedially. Between E10 and E14, the dendrites change their direction of polarization so as to produce the adult pattern.

The axons to the ION play little role in any of these events, because by the time they arrive in the ION (about E12) the first stage of regrowth of the axons is complete. Moreover, tectal lesions at E10-E12 do not cause noticeable effects until after E14, when they are known to cause greatly enhanced neuronal death.

In contrast, the axonal targets seem to play an important role. Early removal of both eye primordia is known to cause the death of virtually all the ION neurons, beginning at E12-E13. But our present data, which are still being quantified, indicate that well before this, by E11, the dendritic trees in the target-deprived IONs are not so highly polarized as in unoperated embryos. This suggests that the axonal target may send an early retrograde signal to the ION neurons that modulates their dendritic geometry before they become dependent on the target for survival.


To determine if trigeminal sensory afferents undergo any reorganization during metamorphosis, ophthalmic (V1), maxillary (V2), and mandibular (V3) nerves were separately labelled with HRP and their central terminal arbors examined in the hind brain and spinal cord of larval and adult frogs. In adults, afferents were arranged in the main sensory nucleus of V with V3 terminals medial and V1 and V2 terminals lateral. Terminals from V3 were also found in an area dorsal to the Vth motor nucleus corresponding to supratrigeminals. The caudal extent of the spinal tract (Vsp) in adults was similar for each peripheral division (V1,2,3). A sparse projection was present at lumbar spinal root 6, but this was overshadowed by the much more dense projections to brachial and thoracic cord levels. Terminals primarily from the mandibular root ended in dense plexuses or tufts in the caudal medulla at the level of the hypoglossal nucleus. These tufts were also present in the contralateral medulla in adults. The basic pattern of sensory afferents was present in stage III larvae including the main sensory terminals and the brachial spinal projections, however, stage III tadpoles contained no Vsp axons caudal to the level of thoracic root 5, no terminals in supratrigeminals, and no projections to the contralateral medulla. These observations suggest that larval development of the trigeminal system includes the expansion of previously existing axons into new terminal fields.

Supported by NIH grants DE 07620 (KEA) and DE 03158 (BMR).

510.9 ABNORMAL CUTANEOUS DIFFERENTIATION FOLLOWING LESIONS OF THE TRIGEMINAL NERVE IN MONODELPHIS PUPS. B.L. Munger* and T.A. Woolsey* (SPON: R.S. Sohn) Division of Surgical Neurology, Neurosurgery & McDonnell Center for Neurological Research, Washington University School of Medicine, St. Louis, MO 63110.

The patterns change after lesions to the whiskers. We used HRP conjugated peanut agglutinin to label whisker representations in the brainstem trigeminal nuclei and the brachial spinal projections, however, stage III tadpoles contained no Vsp axons caudal to the level of thoracic root 5, no terminals in supratrigeminals, and no projections to the contralateral medulla. These observations suggest that larval development of the trigeminal system includes the expansion of previously existing axons into new terminal fields.

Supported by NIH grants DE 07620 (KEA) and DE 03158 (BMR).


When collagen is mixed with dissociated fibroblasts, it is contracted into a dermis-like disk in 1 or 2 days. Keratinocytes can then be seeded on top of this tissue and grow to confluence, forming a skin equivalent (Bell et al., 1981, Science 211: 1052-1054). We have incorporated dorsal root ganglia from 21 day gestation fetal rats into dermal disks at the time of fabrication. The medium used was DMEM with glucose (600 mg/ml), supplements to promote keratinocyte survival (choloragen (10-5 M), hydrocortisone (0.4 ug/ml), and epidermal growth factor (10 ng/ml), plus 10% fetal bovine serum and 10% heat inactivated horse serum. A 40:60 mixture of this medium plus KGM (Clonetics) was also employed. After the dermis was conditioned by the nerves it was seeded with keratinocytes. Three and 6 days later the tissue was fixed for histology and compared to cultures with non-conditioned dermis.

This system may prove useful to test the influence of various cell types on epidermal differentiation. (Supported by PHS grant 5 R23 HD21655-03)

510.11 PEANUT LECTIN STAINING IN THE MOUSE WHISKER-BARREL PATHWAY AND ITS MODIFICATION BY PERIPHERAL LESIONS AT DIFFERENT POSTNATAL AGES. J. Christensen* and T.A. Woolsey (SPON: R.S. Sohn) Division of Experimental Neurology & Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Steindler, Cooper and their colleagues (J. Comp. Neurol. 249:187-189, 1988) showed that appropriately tagged pilot leactin (associated with glia and components of the extracellular matrix) could be used as an indicator of sensory nerve fibers. In the adult whisker representations in the brainstem trigeminal nucleus and V3 in the thalamus for a limited period in early postnatal life. The pattern was altered by lesions to the trigeminal nerve and the brachial spinal projections, however, stage III tadpoles contained no Vsp axons caudal to the level of thoracic root 5, no terminals in supratrigeminals, and no projections to the contralateral medulla. These observations suggest that larval development of the trigeminal system includes the expansion of previously existing axons into new terminal fields.

Supported by NIH grants DE 07620 (KEA) and DE 03158 (BMR).


Adult human muscle fibers cultured aneurally on monolayer are poorly cross-striated, do not contract, and have nuclei located internally. We determined that ImnCMFs are well cross-striated, contract continuously, and most of their nuclei are peripheral. To determine if either innervation or muscle contractile activity alone, or both together, is responsible for the development of cross-striation and the peripheralization of nuclei, we quantitated by light microscopy of 20 fields of 6 sections 1) area covered by cross-striation, and 2) number of peripheral vs. central nuclei, in ImnCMFs contracting for 4 weeks (Contr-ImnCMFs) vs. ImnCMFs paralyzed by tetrodotoxin for 4 weeks (Par-ImnCMFs) from the first day of their contractile activity. As compared to Par-ImnCMFs, Contr-ImnCMFs had: 1) 1400% (p<0.001) more of their total area covered by cross-striations; 2) 510% (p<0.001) more nuclei at the periphery; 3) 70% (p<0.001) fewer nuclei totally. In this culture system, innervation provides an essential signal to induce muscle contraction. However, development of cross-striation and peripheral migration of nuclei, a phenotype of adult muscle fibers, appears to be secondary to muscle contractile activity.
510.13


The purpose of the study is to find out how a deficient length growth of the hindlimb influences the number of axons and the nodal spacing in the rat sciatic nerve. In neonatal rats, the femoral and tibial (phalangeal) collateral nerves on the left side were coagulated with the tip of a microcuret device. Postoperatively femoral and tibial length growth was markedly restricted on the left side, but the foot and pelvic region exhibited a normal longitudinal growth. Following a survival time of 6 months, the sciatic nerves were removed from both sides. Electron microscopic analysis of cross-sections from the nerve revealed that the content of axons was about 20% lower and that the myelinated fibers tended to be smaller on the growth retarded side. Light microscopic examination of teased preparations from pelvic and femoral levels of the left sciatic nerve showed that the relationship between internodal length and fiber diameter was normal in the pelvic segment and that the internodes were abnormally short in the femoral segment. These results suggest that the number of axons in the rat sciatic nerve adapts to a developmental target that sets in at birth, and that internodal elongation during development proceeds according to the local growth in length of the nerve rather than to the length growth of the whole nerve.

510.15


To eliminate developing cholinergic neurons within the nucleus basalis magnocellularis (NBM) region before they become functional (through their terminal points in the neocortex), ibotenic acid was infused bilaterally into the NBM of 2 day-old male and female rats. Behavioral effects were investigated during adulthood and cortical neurochemical determinations were made at sacrifice, one year after lesioning. Histological analysis indicated that lesioned areas were restricted, essentially limited to the globus pallidus (including the NBM), neither the neostriatum nor the thalamus appeared to be damaged. Neurochemically, choline acetyltransferase (CAT) activity was found to be reduced by 25% in the frontal cortex of lesioned animals compared to sham-lesioned controls. Behavioral effects were investigated during adulthood and cortical neurochemical determinations showed significant decreases in the hippocampal size. Furthermore, females indicated significant decreases in the hippocampal size at each dosage of Naltrexone. Male rats in each dosage group showed significantly less than controls for each dosage.

510.16


Six 10-day-old male, unanesthetized, Long-Evans rats were housed in well-ventilated lucite holders and exposed for 17 min. to a 2.3 Tesla field. 9-day-old male and female rats were similarly housed and exposed for 30 min. to a 7.5 Tesla field in a superconducting magnet. Six matched controls for each of the two experimental groups were placed in lucite containers in separate rooms. At 41 days of age, rats were anesthetized, perfused, and brains removed. 20 micro, transverse, frozen sections from the frontal, somatosensory and occipital cortex. On thionine stained sections, cortical thickness measurements of 9 areas were made on micrographs projected (22.5X). All cortical measurements were done "blinded", the codes broken only after all measurements were completed. No significant differences in thickness were noted between the controls and experimental rats exposed to the 2.3 Tesla magnetic field even though 6 out of 9 areas measured were thicker. However, 8 out of 9 areas were thicker in the rats exposed to the 7.5 Tesla magnetic field with 3 areas statistically significantly different: area 4, right hem. (8%; p<0.005); area 10, right hem. (4%; p<0.05); area 3, left hem. (46%; p<0.05). The results suggest that exposure of neonatal rats to high-intensity magnetic fields promotes cortex development.

510.17

REGIONAL EFFECTS OF OPIOID RECEPTOR BLOCKADE ON CORTICAL THICKNESS IN ADOLESCENT RATS. J. Reyes*, G. Lewis*, and M. Diamond (SPON: S. Rooberts). Department of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Studies have indicated that regions of the CNS are increased in size and cellular content when opioid receptors are blocked by Naltrexone. Our study examines the relationship between opioid receptor blockade, cortical thickness, and laterality in 9 regions of the rat cortex in 41-day-olds and 21-day-olds. Our results indicate that the neonatal period is a critical time for the development of both the cerebral cortex and the entire central nervous system. Our findings suggest that opioid receptor blockade may have significant effects on cortical thickness and laterality in the development of the rat brain.

510.18

EFFECTS OF OPIOID RECEPTOR BLOCKADE ON HIPPOCAMPAL DEVELOPMENT IN PREWEANED RATS. D. Fish*, J. Reyes*, T. V. Newlund*, D. Lewis*, and W. C. Diamond (SPON: S. Rooberts). Department of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Collagen synthesis in the CNS has been reported to occur in two circumstances: (1) by the microvessels of the myocardium by Hay, 1971; Treathand et al., 1973) (2) by primary human brain tumors (Rutka, 1987). We have investigated on the presence of collagen in the CNS by using the picrosirius red-polarization method (Junqueria et al., 1979). We visualized the presence of type I collagen in the developing rat brain on days 14.5, 15 and 18 of gestation, and in the newborn rat brain on days 1, 4, and 7. The development of type I collagen was examined by the collagens on days 14.5 and 15 of gestation, by day 1 of postnatal life and by day 4 of postnatal life. Our results show that the CNS tissue and/or the associated mesenchyme synthesize distinct genetic types of collagen in two circumstances; during organogenesis and following injury. The fact that types I and III collagen is often associated to morphogenetic andrepair processes of organs suggest similar roles in the CNS: the setting of pathways for cell migration, the setting of a temporary framework for cell-cell and cell-matrix interactions, as well as the setting of a terrain for the initiation of regeneration and a role to support the growth of axons. In addition, single, thin and green fibers of type III also occupied the neighboring healthy neural tissue. Our results show that type I collagen is the predominant type of collagen during organogenesis and following injury. The fact that types I and III collagen is often associated to morphogenetic andrepair processes of organs suggest similar roles in the CNS: the setting of pathways for cell migration, the setting of a temporary framework for cell-cell and cell-matrix interactions, as well as the setting of a terrain for the initiation of regeneration and a role to support the growth of axons. In addition, single, thin and green fibers of type III also occupied the neighboring healthy neural tissue. The role of these fibers in the development of the CNS is not clear and should be further investigated.

The hypothesis that glial cells can act as immunocompetent cells in the central nervous system has been recently advanced on the basis of in vivo and in vitro studies. However, data on humans are lacking. We have established human fetal brain cultures in our laboratory. Tissues were freshly dissected out of either spontaneous or medically induced abortions, and cells were dissociated and seeded on polylysine pretreated dishes. Cultures were enriched in glial and neuronal cells. Contamination from other cells was minimal. Cells were fixed at different intervals and incubated with monoclonal antibodies against typical markers of B and T lymphocytes and of macrophages. Results showed that cells bore B2, B7, T4-NK, M5, CgB, Thy1-like antigens. Positivity for T8-T9-T11 was lacking. Double staining demonstrated that the most of these antigens were present on GFA-P positive astrocytes. Studies are in progress to test the physiological significance of these data.


The anterior chamber of the eye is a site which readily accepts neural tissue transplants and supplies the transplanted tissue with the hypoxia required for survival. The present study was designed to determine whether neonatal trigeminal ganglion transplants in the anterior chamber of adult rats would innervate the cornea. Transplants survived for at least 6 months after transplant. However, when these transplants were examined with retrograde fluorescein tracing studies and direct examination of corneal innervation using a gold chloride technique, no graft to cornea nerve fibers could be identified. Nor did the grafts innervate the cornea following host trigeminal nerve section. Grafted tissue did innervate the host cornea when the neonatal trigeminal nerve fibers were inserted into the corneal stroma at the time of transplant. The extent to which host trigeminal innervation influences this sprouting is under study currently.

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511.3 TRANSPLANTATION OF ENRICHED CELL POPULATIONS DERIVED FROM IMMATURE RAT RETINA. M. del Cerro and H. B. Yeh. Dept. Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY. 14642.

We and others have successfully transplanted whole immature neural retina. Repaired damaged adult retina, however, may potentially be better approached by selective replacement of neuronal populations. To examine the feasibility of this more refined approach, donor retinal cells derived from selected layers of the immature retina were tested for their ability to survive and differentiate as intraocular transplants. Retinas from P5/Fisher 344 rat pups were isolated, treated briefly with trypsin, rinsed, and plated onto two dishes of mitomycin-treated laminin and then cultured at the level of the developing inner plexiform layer. Each of the two resultant adherent portions, an "outer" portion made up primarily of the neuroblastic mass and an "inner" portion which included the ganglion cells, was detached, suspended in 2 µl of medium and transplanted into the retinas of normal adult Fisher 344 hosts, or a cell layer formed by inner nuclear cell profiles. These observations form transplants consisting of photoreceptor cells, a plexiform layer, and the ganglion cells, was detached, suspended in 2 µl of medium and then cleaved at the level of the developing inner plexiform layer. Each of the two resultant adherent portions, an "outer" portion made up primarily of the neuroblastic mass and an "inner" portion which included the ganglion cells, was detached, suspended in 2 µl of medium and transplanted into the retinas of normal adult Fisher 344 hosts, or a cell layer formed by inner nuclear cell profiles. These observations indicate that selected retinal populations are able to survive and to undergo histogenetic differentiation following transplantation. These enriched cell populations may be a useful source of donor tissue for attempts at repairing damaged retina.

Supported by EY05262 and Rochester Eye Bank.
INTRAOCULAR TRANSPLANTATION OF DEVELOPING RETINA
and Ophthalmology, Univ. of Rochester, Med. Ctr., Roch., NY 14642.

Previous studies were performed using rats as experimental subjects. We have
studied transplantation of developing retina into either normal or damaged retinas,
and that transplantation can be achieved using horseradish peroxidase (HRP)
and antibody against 160 kD Neurofilament. No clear fiber bundles (staining
darkly for the 160 KD Neurofilament (NF)). No clear connections were observedetween the retinal and the tectal graft. Connections to the host inner plexiform layer
even at distances of 1-2 mm. Connections were observed between the retinal and the
tectal graft could be seen. Cerebellar (CB) grafts exposed. A small (~1 mm) incision was cut
through sclera, choroid and retina and closed by 5-0 sutures. Retina from albino rabbit embryos
(15 days after conception) was dissected free from surrounding tissues in cold
bathing medium. Embryonic or neonatal rat retina has been shown to survive and
develop in the host's eyes. Second, S-antigen, a specific marker for photoreceptor cells, is expressed
during the development of transplanted cells. It was seen in transplants examined
at post transplantation (PT) 15 days, but not in those examined only at 3 PT days. The
introduction of a murine system for retinal transplantation opens new possibilities,
particularly through the use of visually defective mutant strains. Supported by
NIH grant 505262 and the Rochester Eye Bank.

RABBIT RETINA TRANSPLANTS TO ADULT RABBIT RETINA:
Eye Research Institute and Harvard Medical School, Boston 02114, and
University of Lund 22185, Sweden.

We report here for the first time the successful transplantation of rabbit embryonic retina to adult rabbit retina, using a modification of the Turner & Blair (1986) technique. Young (4-6 week old) male albino rabbits were used as hosts. Under anesthesia, the dorsal scleral surface of the eye was exposed. A small incision was cut through sclera, choroid and retina and closed by 5-0 sutures. Retina from albino rabbit embryos (15 days after conception) was dissected free from surrounding tissues in cold
bathing medium. Embryonic or neonatal rat retina has been shown to survive and
develop in the host's eyes. Second, S-antigen, a specific marker for photoreceptor cells, is expressed
during the development of transplanted cells. It was seen in transplants examined
at post transplantation (PT) 15 days, but not in those examined only at 3 PT days. The
introduction of a murine system for retinal transplantation opens new possibilities,
particularly through the use of visually defective mutant strains. Supported by
NIH grant 505262 and the Rochester Eye Bank.

NEURON-SPECIFIC MARKERS IN RETINAL GRAFTS TO ADULT RAT RETINA:
B. Ehninger, R. Aramant, M. Seiler, A. Bergström, J.E. Turner and
A.R. Adolph.
University of Lund 22185, Sweden; Eye Research Institute, and Harvard Medical School, Boston 02114; Bowman Gray School of Medicine,
Winston-Salem, NC 27103.

Embryonic rat E15 retinas were transplanted to an adult retinal lesion site as described (Turner & Blair, 1986). Eyes with grafts were fixed with 4%
Paraformaldehyde after survival times of up to 7 weeks. Staining of frozen
sections gave positive results for the following antibodies: CHAT (antibody provided by P.M. Salvadore, CA), TH (antibody provided by P.R. Kozak, NY),
Neurofilament (160 kD), HPC-1 (specific for amacrine cells), and
GABA. Amacrine cell bodies which were positive for CHAT (a cholinergic
marker) could be seen in some 1 week old grafts and in all grafts at later
stages. In 3 and 7 week old grafts, CHAT+ fibers were found in the graft
inner plexiform layer at the border to the inner nuclear layer. TH+
processes of dopaminergic amacrine cells were stained in the graft from 1
to 2 weeks after transplantation. Horizontal cells in the graft stained weakly
for Neurofilament in the glioma layer after transplantation. Vertical processes of host connections made by the graft were blocked by mouse-specific
monoclonal antibodies. The transplants projected only to areas of the brain normally innervated by the eye, including the OPN. The overall density of
innervation did not, however, correlate predictably with the parameters of the
pupillomotor response. Thus ectopic neural xenografts are capable of making specific connections which
are associated with functional innervation, as well as functional connections which
are associated with functional innervation.

COGRAFTS OF RETINA AND TECTUM OR CEREBELLUM TO ADULT RAT RETINA:
Eye Research Institute and Harvard Medical School, Boston, MA 02114

Embryonic or neonatal rat retina has been shown to survive and
differentiate when grafted to an adult retina (see Turner & Blair '86, Aramant et al. '86). Could ganglion cells develop and survive in these grafts deprived
of their target? We wanted to answer this question by transplanting embryonic or neonatal retina to the midbrain of neonatal rats. We transplanted retina from mouse embryos to the midbrain of neonatal rats, which were anesthetized under ether anesthesia at the time of transplantation. After at least 3 weeks the remaining optic nerve was cut and the transplant exposed. Glutaraldehyde
in the transplanted tissue was determined by the density of innervation of the retinal lesion site. At least one specific class of retinal neurons, horizontal cells in the outer plexiform layer, were identified by an antibody against 160 kD Neurofilament.
511.11 RETINAL TRANSPLANTS CAN MAKE FUNCTIONAL CONNECTIONS WITH THEIR MATURE HOST BRAIN. S. Craner*, J.D. Radel and R.D. Lund (SPON: P. Land) Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have previously shown that fetal retina transplanted into neonatal rats are capable of making the connections necessary for driving a pupillary reflex in the host eye in response to light. At birth the rat brain is still developing and therefore presents a favorable environment for fiber outgrowth and synaptogenesis. It is also important to determine whether such transplants will establish functional connections with the normal host retina.

Fetal retina taken from E13 Sprague-Dawley rats were grafted into the pretectal region of 1-day old (PI) Sprague-Dawley albino rats. One of the host's eyes was removed at the time of transplantation. Animals were sacrificed 28 days following transplantation and the presence of a pupillary reflex was confirmed histologically.

This research was supported by NIH grant EY05962 (JDR) and EY05283 (RDL). H. Klassen* and R.D. Lund

511.13 CORTEXAL ACTIVITY CAN BE ELICITED BY LIGHT STIMULATION OF TRANPLANTED EYE. J.D. Radel, J.R. Land (SPON: P. Land) Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previous work in this laboratory has demonstrated that retinal transplants are capable of evoking activity in the suprageniculate thalamus in response to photic stimulation of the transplant. This study examines the ability of retinal transplants to evoke activity in the visual cortex of the host rat.

Retinal transplants were removed from embryonic (E13) rats and transplanted into the midbrain of postnatal (PI) rat hosts. One of the host's eyes was removed at the time of transplantation. Beginning 4 weeks postnatally, the eye was illuminated in an effort to elicit a pupillary reflex in the remaining host eye via the transplant. The right and left visual cortices were then exposed and illuminated with transplanted or normal visual reflexes.

Evoked potentials and multisite activity were recorded in visual areas 18 and 19 of the cortex ipsilateral to the transplant in response to photic stimulation of the transplant. Evoked activity in response to photic stimulation of the transplant was correlated with the appearance of a pupillary reflex in the host eye. These results indicate that retinal transplants can be used to study the effects of photic stimulation on visual cortex activity.

This research was supported by the Eummert Fund of the Pittsburgh Fdn. by NIH grant EY05283 (RDL). H. Klassen* is a Mellon Fellow.


This study tests the potential for visual repair in the host retina by retinal implants. More specifically, it examines the effects of retinal grafts on the local degeneration which follows lesion surgery. Adult Sprague-Dawley rats received a lesion to the retina consisting of a central scotoma. The lesioned rats were sacrificed 10 days after the lesion surgery and the eyes were assayed for responses to photic stimulation.

The results indicate that photic stimulation of retinal transplants is capable of eliciting appropriate activity in the visual cortex, presumably via transplanted visual pathways. The results also indicate that retinal transplants can be used to study the effects of photic stimulation on visual cortex activity.

This research was supported by the Eummert Fund of the Pittsburgh Fdn. by NIH grant EY05962 (JDR) and EYO5283 (RDL). H. Klassen* is a Mellon Fellow.


Retinal transplants have been shown to be capable of driving a pupillary reflex in rats following illumination of the transplant. We investigated whether transplanted retina can also drive the pupillary reflex in the presence of an intact host visual system.

Embryonic (E13) rat retinae were transplanted into the midbrain of one day old rats. In one group of animals the eye was removed at the time of transplantation, while in a second group both eyes were left intact. Beginning three weeks postnatally, the overlying the transplant was removed and the transplant exposed. The results indicate that the pupillary reflex is elicited by photic stimulation of the transplant, although the degree of constriction varied across animals. The transplant-mediated pupillary reflex was observed in all animals in which the eyes were left intact and was in all cases obtainable immediately upon exposure of the transplant. By using 2 separate light sources it was possible to vary independently the intensity of host and transplant illumination. Photic stimulation of the transplant elicited pupillary constriction, the extent of which was directly correlated with the illumination intensity. The results indicate that retinal transplants can be used to study the effects of photic stimulation on visual cortex activity.

This research was supported by NIH grant EY05962 (JDR) and EYO5283 (RDL). H. Klassen* is a Mellon Fellow.
511.17  TRANSLANTATION OF HUMAN PHOTORECEPTORS TO LIGHT DAMAGED RABBIT RETINA. M. Silverman* and W. S. Silverman* (SPON: C. Blazey). Central Institute for the Deaf and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Previously, we have successfully transplanted the human photoreceptor layer from the developing as well as mature rat retina to adult albino rats with light-induced loss of photoreceptors (Silverman and Hughes, Invest. Ophthalmol. Vis. Sci. Suppl. 22:32E; Soc. Neurosci. Abst. 13:306). These results have led us to ask whether mature human photoreceptors from adult eye donors might also be transplantable.

Photoreceptors were taken from the retina of donated human eyes (obtained from the MO Lions Eye Bank and St. Louis Eye Bank) following corneal removal. Hosts were adult albino rats (immunossed or immune competent) expected to cause immunization for photoreceptors which do not cause hyperplasia of the remaining retina intact. The isolated photoreceptor layer was transplanted using a transcorneal approach to the subretinal space. The retina reattaches to the back of the eye with the transplanted photoreceptors integrated into the outer nuclear layer. The transplant stained positive for antismipin antibody RET-P1 (C. Blazey). Identifying the transplanted cells as photoreceptors and further investigating why they may be capable of light transduction, in contrast to transplants in competent hosts shows signs of injection within one week of transplantation. Sham operations showed no repopulation of the host retina with photoreceptors.

These results show that human photoreceptors can be transplanted and, significantly, that mature photoreceptors can be transplanted while other neurons apparently must be developmentally immature for successful transplantation.

Supported by NIH grants EY 04093 and EY 05147 and the Mennosa Company.


Various age rat fetal eyes (e. 14-21d) were removed intact and sutured to the proximal segment of an adult rat sciatric nerve from the same strain. The entire fetal eye/sciatric nerve combination was then inserted into the anterior eye chamber of adult hosts. The distal portion of the sciatric nerve bridge was inserted into the host's forebrain. Simultaneously as the fetal eye/sciatric nerve bridge was implanted, the ipsilateral optic nerve was crushed and dry ice applied to the area of the crush as close to the host retina as possible.

Thirty days post-implantation a 40% solution of HRR was injected intracocularly. In a second group of identically implanted animals the sciatric nerve segment was transected midportion and HRR applied directly to the transected ends. Forty-eight to seventy-two hours after the application of HRR, all animals were sacrificed.

In all host animals, regardless of the age of the fetal eye, the implant survived, grew, differentiated and the labeled patterns in the host forebrain and implant were quantified. This implant/peripheral nerve technique is a useful model for the study of CNS regeneration and repair.


Suppression of pituitary prolactin (PRL) secretion by bromo-cryptine suppresses various immunologic responses in mice. Since cytochrome HC1 (CY5) suppresses pituitary PRL secretion by mechanisms unrelated to dopamine-agonist effects, we examined its effects on immune and neuroendocrine correlates. In a time course study, male C3H/HeN mice received CY5 (200 mg/kg/day; po) and then were sacrificed for determination of serum PRL and corticosterone (CS), and mitogen-induced lymphocyte blastogenesis. Serum PRL levels were unaffected after 1 day of CY5, but were suppressed following 2, 3, and 4 days (46, 51 and 47% of control, respectively). serum PRL remained suppressed 2 and 3 days after CY5 treatment (73 and 52%, respectively). Lymphocyte blastogenesis in response to 1 µg/ml concanavalin A and 2.5 µg/ml lipopolysaccharide were suppressed with 3 or 4 days CY5. Reduced blastogenic responses were noted at 2 and 3 days after stopping CY5 and had recovered by 4 days. CY5 treatment did not increase this level, suggesting that treatment was not a nonspecific stimulant. Co-treatment with PRL (50 µg/day) attenuated immunosuppression in CY5-treated mice. Cytosine treatment also suppressed splenocyte reactivity in a mixed lymphocyte reaction and antibody secretion in a plaque-forming assay. These studies suggest that suppression of PRL secretion mediates the immunosuppressive effects of CY5, further supporting the role of PRL as an important immunotropic hormone.


Recent developments in neuroimmunology have pointed to the interplay between the nervous, endocrine and immune systems. For example, Lymphocytes possess specific sites for a number of neuropeptides, including prolactin and growth hormone.

In the present study, human lymphocytes were cultured with either human or ovine prolactin in combination with the mitogens phytohemagglutinin (PHA) or concanavalin A (Con A). Optimal culture conditions for mitogen stimulation were determined in preliminary studies. Cultures were harvested at 96 or 120 hours; blastogenesis was measured by tritiated thymidine incorporation. In contrast to prolactin or mitogen alone, treatment of cells with PHA or Con A in combination with prolactin resulted in a dose-dependent enhancement of blastogenesis. Both human and ovine prolactin were effective in their additive effects with mitogens. We are currently examining the effects of prolactin inhibitors on alterations of blastogenesis by prolactin.

The data thus far indicate that L-lymphocyte replication in response to mitogens may be influenced by levels of prolactin. These data are in agreement with an earlier report examining prolactin function in the rat (Miestand, et al, Proc. Natl. Acad. Sci., 1986, 83, 1023), and we suggest that prolactin may be a critical neuroimmune modulator.


Immunohistochemistry, Western immunoblotting, and Northern hybridization were used to analyze relative levels of immunoreactive SS and SS mRNA in the brain of immunocompetent neonatal rats, bearing allogeneic-lymphocyte grafts that cause graft versus host disease (GVHD). GVHD levels were compared to those from littermates receiving alloantiserum treatment (AAS) to cure their GVHD or from unimmunized littermate controls (C). The number of SS-immunoreactive cells in the temporal lobe cortex and hippocampus regions were less in GVHD than in AAS or C as were the relative levels of both the neuropeptide and its endogenous mRNAs. Two independent experiments have demonstrated decreased CSF levels of SS results in an increase in the release of hypothalamic corticotrophin releasing factor (CRF), pituitary corticotrophic hormone (ACTH), and corticosterone (CORT). As CORT titers are regulated by SS and CORT is immunosuppressive, we hypothesized that the elevation of SS was related to the decrease in SS in GVHD brain and that the elevated CORT contributes to the profound immuno-deficiency observed in patients with GVHD. SS reverses both the elevation of CORT and the decrease in the number of SS-immunoreactive cells, suggesting that GVHD-relates to the loss of SS-immunoreactive cells rather than cause the loss of SS-immunoreactive cells.

512.4  AGE AND ROUTE SPECIFIC DIFFERENCES IN RESPONSE TO IL-1. L. W. Martin*, L. E. Pester* and J. H. Lipton (SPON: M. Houshian). Dept. of Physiol., The Univ. Tex. Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.

Many aspects of the acute phase response (APR) are muted in the elderly, and the reasons are unknown. Administration of the cytokine IL-1 induces many components of the APR, implicating IL-1 in a mediator of the APR.

To determine if the route of administration or age affect the acute phase response to IL-1 we administered IL-1 intracerebroventricularly (ICV), or intravenously (IV), to young and aged female rabbits. Both intraperitoneal, circulating white blood cells (WBC) and neutrophils, and plasma levels of α-MSH, a neuropeptide that antagonizes actions of IL-1, were suppressed following IV administration of IL-1. ICV IL-1 in young rabbits caused fever, but no significant changes in WBC, neutrophils or α-MSH. ICV IL-1 in old rabbits caused fever and marked increase in neutrophils and α-MSH. IV IL-1 in young rabbits caused fever, increased WBC, neutrophils and α-MSH. IV IL-1 in old rabbits did not cause fever, nor increase WBC, neutrophils or α-MSH. The results indicate that: (1) the response to peripheral and central IL-1 is reduced in aged animals, and (2) that the effect of IL-1 on the APR in young animals is greater when it can act both centrally and peripherally after IV injection.

Supported by WHO GRANT A000109 and NIMDS GRANT NS10046.
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FRIDAY AM NEURAL CONTROL OF IMMUNE SYSTEM III

1215


Recent studies have shown that peripheral blood leukocytes (PBL) can produce POMC-derived peptides, particularly when stimulated by virus or corticotropin releasing factor (CRF). The immunoreactivity of these peptides is not well known in normal subjects and is under investigation.

1216

GLAND TRANSPLANTATION. R.J. Cross and L.Boyarsky, Dept. of Microbiology & Immunology, Univ. of Miami, Miami, Florida. (Supported by USPHS grants NS-9616 and NS-10614.)

This study examined the temporal relationships between stress-induced changes in the neuroendocrine and immune systems. The effects were examined in animals subjected to immobilization or placed in individual cages. Blood samples were drawn at 0, 30, 60, 90, 120, 180 and 240 minutes and coded to prevent contamination. The results showed that stress increased corticosterone and prolactin plasma levels. Basal corticosterone levels (83 ± 31.3 ng/ml) increased 5 fold in the first 10 minutes following surgery. Basal prolactin levels (138 ± 23.3 ng/ml) increased 4 fold by 60 minutes and declined to basal levels within 240 minutes. Basal prolactin levels (12.1 ± 1.9 ng/ml) maximally increased within 30 minutes (141 ± 7) and declined thereafter. The results suggest that opioid peptides play a role in the immune response to stress.

1216

CHEMOTACTIC ACTIVITY OF OPIOID AND NON-OPIOID FRAGMENTS OF BETA-ENDORPHIN. P. Sacerdotto, L.Palazzolo* and A.S.Fenara. Dept. of Pharmacology, School of Medicine, University of Milan, Italy.

B-Endorphin (B-E) has been shown to modulate several immunological functions. Some of these effects are not mediated by a classical opioid receptor, as they are not reversed by naloxone. We analyzed the monocyte chemotactic activity of opioid (N-terminal) and non opioid (C-terminal) fragments of B-E. Both N-terminal and C-terminal fragments are chemotactic. B-E 1-31 is the most potent peak activity at 10^-7 M; C31-43 chemotaxis is reversed by naloxone. The C-terminal fragments bind a receptor different from the classical opioid one. This analysis suggests that B-E can interact with human monocytes at different sites, and suggest a physiological role for the circulating form C-terminal B-E function was still unknown.

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Prostaglandins of the E series (PGE) have been shown to increase cAMP levels, decrease phosphatidylinositol phosphate hydrolysis, and inhibit mitogen-induced proliferation of T-lymphocytes. In brain, B-endorphin (B-E) inhibits cAMP accumulation in rat thymocytes. B-E enhances mitogen-stimulated Ga^-uptake (Hemmick, L.M. and Bidlack, J.M., Life Sci. 41:197 (1987). This study addressed the question: whether opioid peptides reverse the PGE suppression of lectin-induced T-cell proliferation? Cervical and mesenteric rat lymph node cells were cultured for 72 hr with either phorbolmyristin (PMA) or concanavalin A (Con A) in the presence of PGE1 and opioid peptides prior to the measurement of proliferation by 3H-thymidine incorporation into the DNA. BE 1-31 reversed PGE1 suppression of PMA and Con A-stimulated proliferation. The reversal was titratable from 10^-6 M to 10^-4 M, with a maximal enhancement of 1.5 ± 1% at 10^-3 M BE 1-31. [D-ala]-bet-enepehalin (DAHE) also reversed the PGE1 suppression. Neither BE 1-31 nor DAME affected basal or mitogen-stimulated proliferation of T-cells. These data suggest that BE and DAME may regulate T-cell proliferation by counteracting the inhibitory actions of PGE1 and other compounds which stimulate cAMP levels. [This work was supported by grants DA 03742, DA 05302, and DA 07232 from the National Institute on Drug Abuse.]

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We and others have demonstrated that nerve growth factor (NGF) plays a modulatory role in immune function. The mixed lymphocyte response (MLR) is the ability of lymphocytes to recognize and destroy foreign or transformed cells. The autologous mixed lymphocyte reaction (AMLR) is the ability of lymphocytes to be killed as a result of nerve growth factor (NGF) in the MLR only at the highest NGF dose. These results suggest a differential NGF effect on the activation of responding lymphocyte subsets and a response dependent on the type of antigenic stimulus. This work supports our hypothesis that NGF plays a signal role in neuro-immune interactions. Supported by ORR-BR 04108.


Interleukin-1 (IL-1) functions as a mediator of the acute phase of the immune response. IL-1 can demonstrate evoke responses within the CNS as well, including induction of fever, hypothalamic release of corticotrophin releasing factor, and increased slow-wave activity in the sleep EEG. Central effects of IL-1 may be mediated through receptors located throughout the brain (Farrar et al., J. Immunol., 139: 451-463, 1987). We used one- and two-color cultures of rat (IEW) splenocytes (MLR) and autologous T vs non-T cell (LEW) cultures (AMLR). The cells were incubated (5-6 days) either with NGF (0.1-10 ng/ml) and assayed for H-thymidine uptake. In the AMLR increased proliferation was seen for all concentrations of NGF tested. However, a significant increase in DNA synthesis was observed in the MLR only at the highest NGF dose. These results suggest a differential NGF effect on the activation of responding lymphocyte subsets and a response dependent on the type of antigenic stimulus. This work strengthens our hypothesis that NGF plays a signal role in neuro-immune interactions. Supported by MH0621, MH07978.


There is a large body of evidence that sympathetic noradrenergic nerve fibers innervate smooth muscle associated with blood vessels, cardiac muscle, and secretory glands throughout the body. In many instances, neuropeptide Y has been shown to be colocalized within these noradrenergic nerves. Earlier we have demonstrated that noradrenergic nerve fibers, in addition to supplying the smooth muscles of the central arteries and their arteriole branches, form very tight appositions with lymphocytes of the periaiteriolar lymphatic sheath (ILR). We have used electron microscopic immunocytochemistry to localize NPY within the periaiteriolar lymphatic sheath and the lymphocytes. Our findings suggest that the NPY fibers were observed innervating the lateral septum, the bed nucleus of the stria terminalis, the paraventricular nucleus of the thalamus and several regions of the hypothalamus, including the dorsomedial, paraventricular, periventricular and suprachiasmatic nuclei and the lateral hypothalamic area. In the brainstem, TNF-α fibers were found to innervate the central gray matter, the parabrachial nucleus and the doral valse complex. Our findings suggest that the TNF-α neuronal system in the mouse brain may play a role in the autonomic and endocrine responses that occur during the inflammatory response.

512.16 LHb PARTICIPATES IN NEURO-IMMUNE-ENDOCRINE INTERACTIONS. B. Marchetti*, V. Guercio*, M.C. Morale* and U. Scarpagnini* (SPON: M. Motta). Dept. of Pharmacology, Medical School, University of Catania, 95125 Catania, Italy.

The present paper addresses the question of whether the hypothalamic peptide LHb might function as a natural link in the bidirectional regulation of neuroendocrine and immune systems function. Indeed, specific binding sites enabling LHb to act upon the rat thymus gland have been characterized. Thymin LHb binding sites undergo important changes associated with marked modifications of the immune response, namely puberty, castration and aging. Detection of rat anti-LHb antibodies of the IgG class following 15 days in the senes of young and old rats immunized with multiple injections of complete Freund adjuvant and BSA confirmed the profound suppression of the humoral immune response in aging animals, whereas LHb-analog treatment produced a marked increase in anti-LHb antibody titers. Moreover, the in vitro response of thymocytes from both young and old rats to the mitogen concanavalin A (Con-A) was 7-fold higher in LHb-treated animals. In addition, LHb agonists directly potentiate Con-A-stimulated blastogenesis, suggesting that these peptides may also modulate immune system function.
INTERLEUKIN-2 ENHANCES CHICK AND RAT SYMPATHETIC, BUT NOT SENSORY, NEURITE OUTGROWTH. P.K. Haugen* and P.L. Letoumeau, Dept. of Cell Biology, School of Medicine, University of Minnesota, Minneapolis, MN 55455.

An increasing amount of evidence suggests the nervous and immune systems are interconnected. Various neurotransmitters can influence immune responses, and there is evidence that specific membrane receptors are involved in the immune response. This study aimed to determine if IL-2 affects neurons, and secondarily, how sympathetic and sensory neurons from chick and rat respond to LPS in the presence of immune responses.

Neurons from chick and rat DRGs were suspended in cultures without any growth factors. Sensory neurons from chick and rat DRGs were not affected by LPS. In contrast, sympathetic neurons cultured in IL-2 were 200-300% longer than those cultured without IL-2. In chick, the enhancement of neurite outgrowth and length by IL-2 was nearly the same as that seen with NGF. Cells cultured in NGF and IL-2 together did not show an increase in neurite outgrowth, but did increase neurite length over cultures containing NGF or IL-2 alone (chick only). Neuron responses to LPS were dose dependent, with an optimum around 0.2-0.5 ng/ml.

STRESSFUL CONDITIONS ENHANCE AS WELL AS SUPPRESS CELLULAR IMMUNE RESPONSES. A. E. Panerai, and P. Sacerdote, Dept. of Pharmacology, School of Medicine, University of Milan, Italy.

Calcitonin (CT) is a hypocalcemic peptide that possesses a broader range of effects including effects on the Central Nervous System. Recent data suggest that CT may modulate several immune system functions. In this study, we have analyzed the human monocyte chemotaxis activity of different molecular forms of CT, whose activity spectrum in the bone and in the brain are well known. The rank potency order of both the bone and the brain is: eel>CT > salmon CT > human CT, while eel, salmon CTs, and COOP, derived from an alternative RNA splicing, is the principal form and the most active in the brain. On the contrary, eel CT is the less active on chemotaxis (CI=2 at 10^-7 M, salmon is only slightly more potent than human CT (CI=3.5 and 3, respectively) and eel, salmon CTs, and COOP is devoid of any chemotaxis effect. It is possible to modulate the chemotactic activity of the monocyte CT receptor, in fact monocytes of CT treated patients show an impaired chemotactic response to CT suggesting a down regulation of the receptors. These data suggest a possible specific role of CT in the immune system.

DOMINANCE AND IMMUNITY IN NONHUMAN PRIMATES: SOME PILOT OBSERVATIONS. M. Ledan, C. R. Bock*, and B. Muller. Behavioral Immunology Lab, University of Colorado Health Sciences Center, Denver, CO 80204.

The concept of dominance has served as an important heuristic tool for the understanding of social relationships in a variety of species. The present pilot study examines the relationship between dominance ranking in laboratory reared group-housed macaque monkeys and several in vitro measures of the immune response. We found in stable social groups that neither mitogen stimulation nor natural immune responses were increased in the mice that backed out compared to their cage mates. Of the 10 pairs, 9 of the 10 animals that backed out of the tube appeared dominant in the previous five days, 1 trial/day. On Day 15, spleen cells were tested for proliferative responses to ConA and IL-2 were increased in the mice that backed out compared to their cage mates. Of the 10 pairs, 9 of the 10 animals that backed out of the tube on all 14 test days. In PH mice, the same mouse of each pair backed out of the tube on all 14 test days.

ALTERED T-LYMPHOCYTE RESPONSIVENESS IN C3H MICE FOLLOWING A MULTIPHASIC STRESS. J. G. Field, Dept. of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI 48109.

Stressful conditions enhance as well as suppress cell-mediated immune responses. In the present experiment, 6 wk old C3H/HeJ male mice (N=20) were pair-housed and subjected to 1-wk exposure to a light/dark cycle and 1-hr daily exposure to noise. After the light/dark cycle and noise exposure, animals were exposed to either a single exposure of 24-hr fasting or a single exposure to 24-hr fasting and 24-hr exposure to noise. The results of this study suggest a down regulation of the CT receptor, in fact monocytes of CT treated patients show an impaired chemotactic response to CT suggesting a down regulation of the receptors. These data suggest a possible specific role of CT in the immune system.
with a variety of biologic effects. The present study ALTERS THE DISCHARGE FREQUENCY OF HYPOTHALAMIC AND hippocampal neurons. P.M. Dougherty* and N. Dafny (SPON: MICROIONTOPHORETIC APPLICATION OF MURAMYL-DIPEPTIDE MDP was most frequently excitatory in all areas although products derived from immune responses, such as MDP, may the CNS following local application of MDP within the brain regions studied. Among these areas, the hypothalamus. The results obtained from a total of 90 cells of forty male Sprague-Dawley rats, demonstrate a role in neuro-immunologic regulatory pathways during autoimmune disease. These results suggest that MDP may play a role in the mediation of various environmental stimuli into behavior and physiologic processes, rather than simply acting as a neurotransmitter.

513.7
SELF-ADMINISTRATION OF CYCLOPHOSPHAMIDE BY AUTO-IMMUNE MICE. T. Schachman*, J. Myoinah*, L. Grota and R. Ader*. Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14624.

Autoimmune MRL-lpr mice consume more chocolate milk containing cyclophosphamide than congenic +/- mice that are not autoimmune. The hypothesis is that autoimmune mice are able to determine that the immunosuppression resulting from cyclophosphamide is immunotherapeutic for them, while for +/- mice immunosuppression is not adaptive. Male lpr mice but not female lpr mice exhibit this behavior when first exposed to drug at 20 weeks of age. We have examined several factors that influence consumption of cyclophosphamide focusing on differences between lpr and +/- females. Diluting chocolate milk reduces palatability and the ability to mask the flavor of cyclophosphamide but does not discriminate between lpr and +/- females. Drinker measurements revealed that consumption did not discriminate between female lpr and +/- mice when first exposed to drug at 20 weeks of age. Both male and female lpr mice consume more cyclophosphamide than +/- mice when first exposed to drug at 16 and 18 weeks of age. Since the onset of the autoimmune disease occurs several weeks earlier in females than males, these data suggest that the behavioral effect of immunotherapy may depend on the initiation of immunotherapy early in the development of the disease. These data indicate that immune status is able to modify behavior (CNS function) in autoimmune mice.

513.9

Muramyl-dipeptide (MDP) is an endogenous metabolite of gram negative bacterial lipo-polysaccharide (endotoxin) with a variety of biologic effects. The present study investigates whether immunological effects of MDP can elicit a change in the discharge frequency of single neurons in the CNS following local application of MDP within the somatosensory cortex, hippocampus, and hypothalamus. The results obtained from a total of 90 cells of forty male Sprague-Dawley rats, demonstrate a new, specific effect of MDP upon all three brain regions studied. Among these areas, the hypothalamus was most responsive (84%), while the cortex was least reactive (47%). The direction of change induced by MDP was most frequently excitatory in all areas although scattered inhibition and biphasic responses were also seen in the hippocampus. MDP has previously been shown to also play a key role in the integration of various environmental stimuli into behavioral and physiologic processes, and we suggest that the products derived from immune responses, such as MDP, may act directly in the CNS to coordinate autonomic and endocrine function into the systemic response to disease.

513.10

Muramyl-dipeptide (MDP) is the minimal fragment necessary for the biologic activity induced by gram negative bacterial lipo-polysaccharide and endotoxin, and is hypothesized to participate in the mediation of neuro-immune inter-communication. The present study is an investigation of the electrophysiologic activity of single units recorded from freely behaving animals previously implanted with permanent electrodes within the hypothalamus, hippocampus and dorsal raphe prior to and then following increamental systemic (i.p.) dosages of 6-0-Stearoyl MDP. The results obtained from a total of 104 cells of male Sprague-Dawley rats demonstrate that single neurons of the hypothalamus and hippocampus areas previously shown to play a role in the integration of various environmental stimuli into behavior and physiologic processes, and their firing in rather site-specific manners. This specificity includes both the sensitivity as well as the time course of responses to MDP. In contrast, other brain regions such as the dorsal raphe showed little effect following MDP administration. These results suggest that MDP may play a role in neuro-immuno-regulatory pathways during the immune response to bacterial infections.
EVALUATION OF SUPPRESSED LYMPHOCYTE RESPONSIVENESS
INDUCED BY NEURAL CONTROL OF IMMUNE SYSTEM IV

AND INDEPENDENT PATHWAYS. J. E. Cunnick*, D. T. Lysle*, of Clin. Immunopath., Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15213-3417.

Presentations of 16 signaled foot-shocks is capable of suppressing the immune response to concanavalin A (Con A). This suppression was not due to changes in total leukocytes, or the percentage of T cells, T-helper cells, or T-nonhelper cells as determined by flow cytometry. Although the addition of recombinant IL-2 stimulated the mitogenic response of splenocytes from shocked and control rats, the response of shocked splenocytes remained suppressed in comparison to all controls. Splenocytes from shocked rats produced normal amounts of IL-2 in response to Con A stimulation. Preliminary analysis of IL-2 receptors on the stimulated lymphocytes showed no quantitative differences between shocked and control rats. Splenocytes from shocked rats also demonstrated a suppressed incorporation of [3H]-thymidine when stimulated with calcium ionophore A23187.

In conclusion, shock-induced suppression of mitogenic responsiveness was not due to changes in lymphocyte subpopulations or IL-2 production. However, a calcium dependent biochemical pathway may be associated with the suppressed T cell responsiveness.

STRESS AND BRAIN REACTIVE AUTOANTIBODY LEVELS IN MURINE AUTOIMMUNE MODELS OF SLA. A. Narendran*, S. Harkins*, S. A. Hoffman, Dept. of Microbiology, ARD, Tucson, AZ 85727.

Psychosocial factors are known to interfere with immune system functioning. Conversely, abnormal immune activity may also be implicated in CNS dysfunction. As part of ongoing research into the nature, function and regulation of pathogenic anti-brain autoantibodies, we have investigated the effect of stress on the production of such molecules.

Animals from 4 autoimmune strains (MRL/L, BXSB, HDW/N and NZB) were exposed to normal saline or to a non-autoimmune strain (Balb/c or C57BL/6) were divided into two age and sex matched groups, and NZB) and 2 non-autoimmune strains (Balb/c and C57BL/6) were divided into two age & sex matched groups. Splenocytes from shocked rats produced normal amounts of IL-2 in response to Con A stimulation. Preliminary analysis of IL-2 receptors on the stimulated lymphocytes showed no quantitative differences between shocked and control rats. Splenocytes from shocked rats also demonstrated a suppressed incorporation of [3H]-thymidine when stimulated with calcium ionophore A23187.

In conclusion, shock-induced suppression of mitogenic responsiveness was not due to changes in lymphocyte subpopulations or IL-2 production. However, a calcium dependent biochemical pathway may be associated with the suppressed T cell responsiveness.


Acute stressors contribute to behavioral and neuroendocrine consequences of stress exposure. In the present study the influence of such factors on immune reactivity following stress was investigated. Because of the complexity of the relationship between stress and immunity, neuroendocrine activity was also assessed.

After stress exposure, mice of several inbred strains were sacrificed for determination of splenic Natural Killer (NK) cell cytotoxicity and CNS. The major finding was that activity was significantly suppressed in rats exposed to restraint stress, and that the magnitude of the stress-induced suppression varied among inbred strains. For example, a significant reduction of NK was noted in C57BL/6 mice 24-48 hr after stress, whereas among C57BL/6 mice activity was reduced within 30 min. of stress onset. Catecholamine alterations engendered by stress also varied across strains.

This neuroendocrine reactivity was apparently more pronounced in those strains which exhibited a larger or more rapid diminution of NK cell activity after stress.

These data indicate that there are significant strain-specific variations in the response of the immune system to stressors. Furthermore, they provisionally suggest that alterations in catecholamine activity may contribute to the effects of stress on immune function. (Supported by NSERC Grant U05697)


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513.15 SSRS AND BRAIN REACTIVE AUTOANTIBODY LEVELS IN MURINE MODELS OF SLA. A. Narendran*, S. Harkins*, S. A. Hoffman, Dept. of Microbiology, ARD, Tucson, AZ 85727.

Psychosocial factors are known to interfere with immune system functioning. Conversely, abnormal immune activity may also be implicated in CNS dysfunction. As part of ongoing research into the nature, function and regulation of pathogenic anti-brain autoantibodies, we have investigated the effect of stress on the production of such molecules.

Animals from 4 autoimmune strains (MRL/L, BXSB, HDW/N and NZB) were exposed to normal saline or to a non-autoimmune strain (Balb/c or C57BL/6) were divided into two age and sex matched groups, and NZB) and 2 non-autoimmune strains (Balb/c and C57BL/6) were divided into two age & sex matched groups. Splenocytes from shocked rats produced normal amounts of IL-2 in response to Con A stimulation. Preliminary analysis of IL-2 receptors on the stimulated lymphocytes showed no quantitative differences between shocked and control rats. Splenocytes from shocked rats also demonstrated a suppressed incorporation of [3H]-thymidine when stimulated with calcium ionophore A23187.

In conclusion, shock-induced suppression of mitogenic responsiveness was not due to changes in lymphocyte subpopulations or IL-2 production. However, a calcium dependent biochemical pathway may be associated with the suppressed T cell responsiveness.

STRAIN DIFFERENCES IN IMMUNORESPONDING TO STRESS. J. Irwin. Dept. of Psychology, University of British Columbia, Vancouver, BC V6T 1Z2.

An effort to identify autoantibodies associated with various neurologic and psychiatric diseases discovered sera from normal human serum. These data indicate that there are significant strain-specific variations in the response of the immune system to stressors. Furthermore, they provisionally suggest that alterations in catecholamine activity may contribute to the effects of stress on immune function. (Supported by NSERC Grant U05697)

513.16 NATURALLY OCCURRING AUTOANTIBODIES IN HUMAN SERUM: REACTIVITY WITH CENTRAL NERVOUS SYSTEM PROTEINS. J.S. Frazier* and D.M. Jacobsowitz (Spons: W. Heydorn), Lab. of Clin. Sci., NIMH, Bethesda, MD 20892.

Antibody is being recognized as a major component, if not cause, of an increasing number of diseases including those of the CNS. In an effort to identify autoantibodies associated with various neurologic and psychiatric diseases it was discovered that sera from normal human serum contain autoantibodies which react with a number of CNS proteins. Two-dimensional polyacrylamide gel electrophoresis was performed on 100 µg of normal human serum. Proteins on the resulting gels were Western blotted onto nitrocellulose membranes. Non-specific membrane binding sites were blocked with 5% BSA (per spot). The blots were incubated individually with sera from 20 normal controls at dilutions of 1:10 to 1:1000. Bound autoantibodies were visualized by the methods of Towbin. Reactivity against a number of CNS proteins was observed in a large percentage of sera. These proteins included, among others, neurofilaments, synaptophysin, glial fibrillary acidic protein (GFAP), the 8-subunit of the G protein, serum glutamic oxaloacetic transaminase (SGOT), actin and tubulin. The identity of these proteins was confirmed by comparing the location of the blot-spots with the locations of spots produced with antibodies specific for these proteins. Antibodies against these proteins may be important in the normal and the diseased state.

513.17 NEURAL CONTROL OF IMMUNE SYSTEM IV 1283 FRIDAY AM NEURAL CONTROL OF IMMUNE SYSTEM IV
514.1 FACTOR BINDING TO A CRF-INDUCIBLE ELEMENT OF THE RAT POMC GENE

Expression of the gene encoding proopiomelanocortin (POMC), the precursor of several pituitary peptide hormones including ACTH, ß-endorphin, and ß-lipoprotein, has been previously shown to be positively regulated by the hypothalamic peptide CRF, both in vivo and in primary anterior pituitary cultures in vitro. We have found that treatment of the AtT20 mouse pituitary cell line with CRF for 60 minutes increases the POMC gene expression by 2 fold, and after treatment with CRF for 24hrs a 2-3 fold increase in POMC mRNA levels is observed. By constructing a series of deletion mutants of the rat POMC 5' flanking DNA a hormone responsive promoter, we have shown that chromoharacteristic acetyl transfer (CAT) reporter gene followed by transfection into AtT20 cells, we have identified a fragment within the POMC flanks that confer CRF and forskolin inducibility on the TK promoter (334 to 133). Analysis of the region of the rat POMC promoter between -478 and -350, an area which appears partially responsible for the elevated basal activity of the POMC promoter by transient transfection assays, using gel shift studies, deletion and methylation interference studies, shows several sites protected by factors present in nuclear extracts of AtT20 cells. Studies are currently underway to determine the hormone inducibility and cell/line distribution of these DNA-binding factors.


It has been previously shown that chronic treatment with dexamethasone (DM) decreased glucocorticoid binding capacity in the rat anterior pituitary, however this decrease in binding was not observed after chronic stress. Data obtained from the present studies suggests that chronic stress may reflect the involvement of other factors in regulation in the pituitary.

To further examine CRF regulation in the rat anterior pituitary, we have analyzed cytoplasmic mRNA levels after acute and long term CRF treatment, as well as glucocorticoid regulation of CRF treatment. For acute stress adult female Sprague-Dawley rats (n=20) were injected s.c. with 20 µg of CRF or vehicle and sacrificed after 30, 60, and 4hrs, in chronic studies, animals were injected twice daily for 7 days. To determine CRF gene transcription rate, an in vivo nucleic run-on assay was used; cytoplasmic mRNA levels were quantitated in a solution hybridization/nuclease protection assay using an antisense RNA probe.

A 2.4 fold increase in GR gene transcription was observed at 30 and 60 min after CRF administration, but had returned to control levels after 4hrs. Cytoplasmic GR mRNA levels were significantly above control at 4hrs. After CRF treatment, chronic stress (7 days) failed to show any change. To determine if the CRF response is a direct effect or is mediated through ACTH, stimulated release of glucocorticoids we are currently examining the effects of CRF and glucocorticoids on GR mRNA levels and GR gene transcription in primary cultures of rat anterior pituitary, as well as in AtT20 cells.


Phosphoprotecine (POMC) producing cells comprise nearly 100% of the adult intermediate lobe hormone producing cells. Secretion by these cells is primarily under negative regulation by dopamine. Although the POMC-derived peptide α-MSH has been injected in plasma of fetal rat and lambs, no study thus far has directly examined the secretory capabilities of fetal melanotrophs. Here we have utilized the reverse hemolytic plaque assay, an assay that is highly sensitive to basal and regulated release by melanotrophs at fetal and early post-natal ages. Only basal secretion was detected at the earliest ages examined (e17.5). CRH (10-7M) stimulated secretion was observed at e19.5 and continued through postnatal ages; incubation with CRH (10-7M) during this time period increased both the plaque size and the percentage of melanotrophs stimulated to secrete. Dexamethasone (10-7M) inhibition of CRH (10-7M) stimulation was detected from e19.5 to p2. At p3 dexamethasone no longer inhibited melanotroph secretion although inhibition of CRH-stimulated release in p3 corticotrophs was readily demonstrable. Dopamine (10-7M) inhibition secretion at the earliest ages studied (e17.5); this effect persisted throughout all ages examined (e17.5-p2). These results suggest that melanotrophs appear to undergo a maturation process which includes the loss of the alpha-MSH receptor as CRF (e17.5), next poses both functional CRH and steroid receptors (e19.5) and finally undergo loss or uncoupling of steroid receptors (p2). The loss of steroid-inhibited phosphorylation (p3) is closely coupled with the arrival of catecholaminergic input into the neurointermediate lobe (p2). However, the early response of melanotrophs to dopaminergic agonists, which can be detected seven days prior to arrival of catecholaminergic fibers into the neurointermediate lobe, appears to be an intrinsic feature of these cells that is never present in corticotropic. Supported by HD-18992.


The intermediate lobe (IL) of the rat pituitary is primarily melanotrophs which are organized into clusters of cells called lobules. Previous culture systems using dispersed IL cells may not represent cellular behavior in vivo accurately since earlier data from our laboratory have shown that melanotrophs growing in monolayer exhibit a higher proliferation rate compared to the IL in vivo. To study both the regulation of proposi-melanocortin (POMC) biosynthesis and IL proliferation, we have recently developed a primary culture system of rat IL cells that retain both dispersed intact IL lobules. The effects of CRF, GABA and quipazine (SHT2 agonists) on the synthesis of POMC mRNA, release of immunoreactive ß-endorphin (iß-END) and IL proliferation were studied using this model.

CRF increased the amount of ß-END secreted at both a 10-9 and 10-7 M dose. Both concentrations of CRF augmented the relative levels of POMC mRNA although the increase was statistically significant only with the higher dose. Quipazine also increased the levels of POMC mRNA significantly at both the low (10-8 M) and high (10-6) doses. Total ß-END secreted into the media was increased only with the higher dose of the two compounds. No alteration in proliferation was seen with either compound. Inclusion of GABA in the media at concentrations up to 10 mM as well as muscimol and bicuculline failed to produce a detectable alteration in the levels of POMC mRNA; ß-END secretion and proliferation rate. In conclusion, CRF and SHT2 agonists not only increase the release of POMC-derived peptides from mechanically dispersed IL lobules but also increase the levels of POMC mRNA. On the other hand, GABAMetabolites did not appear to affect mRNA levels nor ß-END secretion.


Release of POMC (α-MSH and ß-endorphin) peptides from the pituitary pars intermedia is regulated by both neurotransmitters and neuroendocrine input. Dopamine (DA), an inhibitor of POMC release from pituitary lobes, was used to examine the corticotropin-releasing factor (CRF)-induced stimulation of peptide secretion in vivo (Saland et al, '87, Soc. Neurosci. Abst. 13: 418, 1b) and in primary cultures of rat intermediate lobe (NILS) incubated for up to 120 minutes in BICOMB media containing glucose, glutamine, 0.1 mM bacitracin, 0.1 mM ascorbic acid, and 10 µM epi-butyryl-D-Ala2-Met5-enkephalinamide (DALA, 10-6) or Sandoz (SAN) gynkopeptide (10-7M) were added, with or without DA (10-6) or naloxone (NAL, 10-7). DALA or SAN plus DA transduced increased POMC release above basal levels in the presence of DA alone at some, but not all time points. NAL alone reduced α-MSH release, while addition of NAL to peptides plus DA induced POMC release above control levels in several incubations. POMC peptides were measured by radioimmunoassay (RIA). Immune staining of tissues for POMC peptides and EM cytology of NILS correlated with POMC mRNA levels. In contrast to dopamine, naloxone, or nilactone, are found on intracellular vesicles. Supported by NH 21256 and RR 04193 (LCS).
ATHC secretion by the anterior pituitary is stimulated by several humoral and neural pathways, including corticotropin-releasing factor (CRF), vasopressin (AVP) and catecholamines. In the present studies, we have examined ACTH release from rat pituitary tissue using a range of concentrations in hypophysial-portal plasma from rats. At levels approximating endogenous molar ratios, these secretagogues were tested for their ability to stimulate ACTH release. To more closely mimic the response found in vivo, freshly removed rat anterior pituitaries were used in a superfusion system. At concentrations well below the maximal 6-fold increase in the release of ACTH, the EC50 for the CRF-induced ACTH release was 12 nM. At concentrations above 300 nM AVP elicited a maximal 1.7-fold increase in the release of ACTH. The EC50 for the AVP-induced ACTH release was 7 nM. At concentrations above 1 µM the catecholamines epinephrine (E) and noradrenaline (NE) elicited a maximal 2.6-fold increase in the release of ACTH. These results suggest that: 1) an intact ACTH release by 45% (p<.001: determined by Kruskal-Wallis ANOVA). This effect was manifested after 3 hours, an elevation of ACTH is seen. The combination of CRF and E is less than additive across the full physiologic range of the secretagogues with a maximal 7.3-fold increase of ACTH release. Mimicking the increased CRF and AVP concentrations observed in hypothalamic-portal plasma during hemorrhage, 150 µM and 800 pM respectively, a similar 2.3-fold elevation of ACTH is seen. The combined effect of CRF and E is less than additive with a maximal 7.8-fold increase in ACTH release. This is the first report of effects of endogenous portal levels of secretagogues on ACTH secretion in vivo. The results support the validity of this particular preparation as a model of stress-induced ACTH release.

514.9

MOLECULAR MECHANISMS CONTROLLING POMC GENE EXPRESSION. Neil M. Martin, Kyriakos T. Terzis and Terry Reiss. Dept, of Pharmacology, Univ. of Pennsylvania, Phila., PA 19104. The control of POMC gene expression is an important event in the body's response to stress. The molecular events controlling POMC gene expression are currently unknown. Since several ligands are known to both increase POMC mRNA levels in the anterior pituitary and activate protein kinases, it is possible that phosphorylation is a key event in the control of POMC gene expression. We have examined the effects of corticotropin-releasing factor (CRF), which is known to activate CAMP-dependent protein kinase, on phosphorylation of nuclear proteins. Using AT-T20 cells, a mouse anterior pituitary cell line, we have found at least three nuclear proteins which are phosphorylated in response to CRF (10-7 M). These proteins, visualized using two-dimensional gel electrophoresis and autoradiography, have molecular weights of 45,49, and 56 kilodaltons and pi values between 5.9 and 6.4. Protein phosphorylation has been shown to be enhanced within 5 minutes of CRF treatment. It is possible that these proteins interact with upstream elements of the POMC gene to cause an increase in DNA transcription. Work is currently being done to examine the effects of phorbol esters and calcium ionophores, which increase POMC mRNA levels and can activate distinct protein kinases, on phosphorylation of nuclear proteins. Supported by NIH grant DK37404 and American Heart Association grant-in-aid.

514.11

α-ANF[1-28] BUT NOT α-ANF[5-28] INHIBITS CRF-STIMULATED ACTH SECRETION FROM CULTURED ANTERIOR PITUITARY CELLS. H.S. King* and A.J. Baertschi. Neuroscience Program and Department of Physiology, University of Virginia, Charlottesville, Va. 22908. The effectiveness of α-anterior pituitary factors (ANF) as inhibitors of ACTH secretion was examined in dispersed rat anterior pituitary cells that had been in culture for 4 days. Eleven experiments were conducted with 24-well plates using 4 wells/combination of peptides. α-ANF[1-28] significantly inhibited ACTH release stimulated by 1 nM CRF. At concentrations of 10 to 1000 nM, α-ANF[1-28] inhibited ACTH release by 45% (p<.001: determined by Kruskal-Wallis ANOVA). An effect was manifested after 3 hours but not 0.5 or 1 hour, of incubation suggesting that ACTH synthesis may have been reduced by α-ANF[1-28]. Conversely, at concentrations of 10 to 10,000 nM, α-ANF[5-28] had no effect on ACTH secretion after 0.5, 1 and 3 hours. These results suggest that: 1) an intact secretory process is required for inhibition of ACTH. These requirements may reflect a previous failure to demonstrate inhibition of ACTH by ANF. Thus, α-ANF[1-28] may be a physiological inhibitor of ACTH secretion. (Supported by University Technology Corporation and the Virginia Center for Innovative Technology).

514.8

EXPRESSION OF BIOLOGICALLY ACTIVE RAT CORTICOTROPIN-RELEASING FACTOR (CRF) IN A TRANSFECTED MEDULLARY THYROID CARCINOMA (MTC) CELL LINE. G. Hammer*, V. Falchicchi*, E. Serarrin*, and J. Low*. (SPON: R. Ventsimigli). Neuroscience Graduate Program, Tufts U. School of Medicine and Div. of Molecular Medicine, New England Medical Center, Boston, MA 02111. CRF is a neuropeptide that acts as both a hypophysiotrophic regulator of ACTH secretion and a mediator of central autonomic responses to stress. Studies of the biosynthesis of CRF in humans have been limited by the lack of a suitable culture system. To provide such a system, we have engineered clonal cell lines that express high levels of rat CRF. W2 cells, a transplantable rat MTC cell line (provided by B. A. Ross, Univ. of Wash., Seattle, WA), were cotransfected with a CRF cDNA (provided by R. Thompson, Oregon Health Sciences Univ., Portland, OR) driven by a cytomegalovirus immediate early promoter/enhancer and a neomycin resistance p < 50% transfection driven by an RSV promoter. These cells were chosen as the best cells because of their high concentrations of secretory granules and their ability to process and secrete neuropeptides. Media from 514.8-resistant clones were screened by radioimmunoassay to detect CRF expression. The transfected, but not the wild type cells, secreted assayed CRF. To assess biological activity of the immunoreactive CRF, dispersed primary pituitary cultures were treated with conditioned media from the 6 clones secreting the highest concentration of CRF (< 50 µM). ACTH release from the pituitary cells was stimulated identically by treatment with either synthetic CRF-amide or transfected W2 cell conditioned media. In conclusion, we have made stably transfected cell lines that express rat CRF. The secreted immunoreactive CRF is biologically active suggesting that the pro-CRF produced is correctly processed and matured. Studies are in progress to characterize the biosynthesis of CRF in these cells further and to use them, via surgical implantation, as an ectopic source of CRF in transplantable rats.
A PRACTICAL RADIOIMMUNOASSAY FOR PLASMA CORTICOTROPIN -
RELEASEING FACTOR (CRF). The primary antisem for this
assay was produced in rabbits against rat neurocrine CRF coupled
to hemocyanin via glutaraldehyde. The antisem is used at a
dilution of 1 to 30,000. Radiolabelled CRF is prepared by
chloramine T iodination of Tyr^1-CRF and is purified by
HPLC. Samples for assay are drawn into cold EDTA coated
plastic syringes containing 0.1 ml of an enzyme killer
solution per 1 ml of whole blood. Plasma is extracted using
two Sep-Pak Cartridges (Analyt. Inc. Newark, DE) hooked
in tandem. Standard curves are prepared in CRF-free plasma and
extracted as samples. Recovery averages 80% by trace-
recovery sampling. The assay was able to detect 40 pg/ml
addition and second antibody technology to improve sensi-
tivity. The minimal detectable quantity for the assay is
2.5 pg/ml and resultant data were analyzed using SigmaStat 7,
80, 900 pg/ml respectively. No cross reactivity is detect-
able with ovine CRF up to 1ug/ml. Cross reactivity with other
neuropeptides is negligible.

Data concerning baseline concentrations, physiologic and
physiochemical validations of the assay will also be
presented.

PLASMA ACTH IN THE RAT DEMONSTRATES THREE DISTINCT
RHYTHMS WITH 24 HOURS. M. Sarabi, J.L. Lent", S. Fezzi* and D.K. Hazel*, Wm. S. Middleton Veterans
Hospital and Dept. of Medicine, University of Wisconsin,
Madison, WI 53705.

ACTH secretion by the pituitary is rhythmic and
episodic, as reflected by fluctuations in plasma
concentrations of ACTH. The present work was de-
signed to further characterize the patterns of ACTH
secretion occurring simultaneously within a 24-hour
period in the rat. Blood sample collection proto-
cols with sampling intervals of 10 minutes, 15
minutes, and 4 hours were used in awake, chroni-
cally cannulated rats. Plasma samples were assayed
for ir-ACTH and resultant data were analyzed using
the PULSAR program after determination of appropriate
constants. The distinct patterns of ACTH secre-
tion were demonstrated within a 24-hour period.
In addition to the circadian variation with peak plasma
ACTH levels occurring one hour before lights-out, plasma ACTH exhibited episodic ultradian vari-
ation of two types: 11-19 pulses in 24 hours, which we have called the "larger ultradian" pulses, and shorter episodic bursts occurring approximately 3 times per hour, which we have called "micro-
pulses."

EFFECT OF ALPHA-MELANOCYTE STIMULATING HORMONE
(αMSH) ON TUBEROHYPophysial, TUBEROINFUNDIBULAR, AND NIGROSTRITAL DOPAMINERGIC NEURONAL ACTIVITY.
S.E. Lindley*, R.J. Lookingland and K.E. Moore, Dept. of
The purpose of this study was to determine if αMSH feedback
regulates its own secretion by altering the activity of
tuberohypophysial dopaminergic (THDA) neurons
in the intermediate lobe (IL) of the pituitary. Accordingly, the
effect of intracerebroventricular (icv) administration of αMSH
was examined on the activity of THDA neurons. For comparison,
the effect of αMSH was also examined on the activity of
tuberoinfundibular dopaminergic (TIDA) neurons terminating
in the median eminence and nigrostriatal dopaminergic (NSDA)
neurons terminating in the striatum. The activity of DA neurons
was estimated using biochemical techniques. Administration
of αMSH (20 μg, icv) to male Long-Evans rats did not alter:
1) the concentrations of dihydroxyphenylacetic acid (DOPAC),
2) the rate of DA turnover, and 3) the rate of DA synthesis in the
IL or striatum. These results indicate that exogenously
administered αMSH does not alter THDA or NSDA neuronal
activity. In the same animals it was found that αMSH increased:
1) the concentration of DOPAC, 2) the rate of DA turnover, and
3) the rate of DA synthesis in the median eminence, thus increasing
the secretion of prolactin. (Supported by NIH grant NS 15911)

EVIDENCE THAT ACTIVATION OF THE HPAA BY SINGLE-DOSE ADMINISTRATION OF ETHANOL IS NOT MEDIATED BY MEDIAN
EMINENCE AVP OR ADRENAL CATECHOLAMINES. A.B. Thiagarajan* B.L. Erskine* (Spon: S.K. Katz), LCS/NIAAA, National Institutes of
Health, Bldg 10, Room 3C-218, Bethesda, MD 20892.
Single-dose ethanol (Et) administration activates the
hypothalamic-pituitary-adrenal axis (HPAA) as monitored
by enhanced levels of adrenocorticotropic (ACTH) hormone,
gluocorticoids (Corticosterone, Co) and adrenomedullary-
derived epinephrine (Epi); however, an understanding of
the precise mechanism or site of ethanol's activation of
the HPAA remains incomplete. In order to determine if
median eminence (Me)-derived vasopressin (AVP) or adrenal
Epi are partial mediators of Eth-induced ACTH plasma levels, an
in vivo microdialysis procedure was employed. Two
pairs of rats were intraperitoneally administered saline
derived Epi as partial mediators of Eth-induced ACTH plasma levels. G. A.
Cudelsky, S. Berry* and H.Y. Meltsner, Case Western
Reserve University, Cleveland, OH 44106.

In this study, we will describe an RIA for plasma Corticotropin -
Releasing Factor (CRF). The primary antiserum for this
assay was produced in rabbits against rat/human CRF coupled
to hemocyanin via glutaraldehyde. The antiserum is used at a
dilution of 1 to 30,000. Radiolabelled CRF is prepared by
chloramine T iodination of Tyr^1-CRF and is purified by
HPLC. Samples for assay are drawn into cold EDTA coated
plastic syringes containing 0.1 ml of an enzyme killer
solution per 1 ml of whole blood. Plasma is extracted using
two Sep-Pak Cartridges (Analyt. Inc. Newark, DE) hooked
in tandem. Standard curves are prepared in CRF-free plasma and
extracted as samples. Recovery averages 80% by trace-
recovery sampling. The assay was able to detect 40 pg/ml
addition and second antibody technology to improve sensi-
tivity. The minimal detectable quantity for the assay is
2.5 pg/ml and resultant data were analyzed using SigmaStat 7,
80, 900 pg/ml respectively. No cross reactivity is detect-
able with ovine CRF up to 1ug/ml. Cross reactivity with other
neuropeptides is negligible.

Data concerning baseline concentrations, physiologic and
physiochemical validations of the assay will also be
presented.
REDUCTION OF ACTH-LI IN PLASMA FOLLOWING T.V. INJECTION OF DELTA SLEEP-INDUCING PEPTIDE (DELTA SLP) IN MEN. B. L. Desjardins, L. M. Pekarek, S. Bergquist, E. Widderick*, Dept. of Psychiatry and Neurochemistry, Univ. of Lund, P.O. Box 630, S-220 06 Lund, Sweden.

Recent studies have indicated that one component of Delta sleep-inducing peptide (DSIP) in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. We have now examined how DSIP affects the plasma levels of corticotrophin-like immunoreactivity (ACTH-LI), cortisol and some other neuropeptides related to the HPA axis in man. The present study is a double blind-cross-over challenge test in which eleven healthy male volunteers (age 25-29) achieved a single dose of synthetic DSIP (2 mmol/kg b.w.) intravenously (i.v.) during 6 min. An equal volume of saline served as control. The plasma conc. of DSIP increased approximately three times (mean) at 5 min after DSIP inj. and was then rapidly normalised. ACTH-LI in plasma decreased in all but one of eleven individuals and the mean conc. of ACTH-LI in plasma was significantly reduced at 5-180 min after DSIP inj. The controls showed an increased ACTH-LI level. No differences were found in cortisol. Urinary analyses of stress parameters like cortisol and mononuclear metabolites did not reveal any differences either. In conclusion: A single dose of DSIP significantly reduces the plasma conc. of ACTH-LI in man and may thus be yet another factor involved in the regulation of ACTH secretion in man.

THIOTROPIN-RELEASE-ING FACTOR (TRF) EFFECTS ON PITHYRIAL ACTH BIODISTRIBUTION AND IMMUNOREACTIVE FUNCTION. J. Rivier2 and T.S. Gray2. Dept. of Medicine and Surgery, Univ. of Calif., San Diego Medical Center, San Diego, CA 92103; 2Dept. of Anatomy, Loyola Medical Center, Maywood, IL 60153.

TRF, similar to corticotropin-releasing factor (CRF), acts within the brain to increase plasma concentrations of catecholamines (CA), and mean arterial pressure (MAP) and heart rate (HR). The goal of these studies was twofold: 1) to determine if TRF, similar to CRF, given intracerebroventricularly (icv) would increase plasma cortisol (CRF) concentrations; 2) to determine if the CRF-like actions of TRF could be antagonized with the CRF-receptor antagonist, α-hel CRF 9-41. Experiments were performed in awake animals equipped with chronic right atrial, femoral artery, or icv cannulae. In vivo experiments, icv doses of ACTH and CRF were measured using established methods. TRF (0.001 μg, 2.6 nmol) or CRF (2 nmol) given icv produced increases in plasma concentrations of ACTH and CA, and of MAP and HR. TRF-induced elevation of plasma ACTH levels was dose related and did not occur following iv administration of TRF. α-hel CRF 9-41, at a dose (12 nmol) that inhibited CRF-induced (2 nmol given icv) elevation of plasma levels of ACTH and CA, and MAP and HR, did not modify TRF's actions. Since TRF does not act directly on the pituitary or via CRF release to stimulate ACTH secretion, alternative mechanisms exist, e.g. vasopressin, CA, or unidentified factors, to mediate the observed ACTH release.


Cholinergic neurons in basal forebrain degenerate in Alzheimer's Disease (AD). Both arginine vasopressin (AVP) and corticotropin-releasing factor-containing neurosecretory cells are innervated by cholinergic neurons, some of which probably originate in the basal forebrain. In an effort to understand the role of cholinergic mechanisms in the regulation of neuroendocrine systems, the effects of the cholinesterase inhibitor physostigmine (0.0125 mg/kg iv) in 12 men with AD and compared responses to 12 age-matched normal men.

Physostigmine promptly increased plasma AVP (10 fold), E (2-3 fold) and EPI (3 fold) in elderly controls. In contrast, plasma AVP and EPI responses to physostigmine were significantly lower in AD patients and control responses to physostigmine. Differences were most pronounced when control and AD patients who experienced nausea (m2 and 6, respectively) were included. Expressed as area under response curves, AD patient AVP (2±1.2 pg/ml/min) and E (5±1.5 pg/ml/min) were significantly (p<0.02) less than those of controls (14±5 and 28±4 pg/ml/min, respectively). AD EPI (10±3 pg/ml/min) tended to be lower than that of controls (60±7 pg/ml/min; p<0.1).

We conclude that the cholinergic deterioration of AD also influences CNS regulation of neuroendocrine systems.
NEUROPSYCHOLOGICAL CORRELATES OF BILATERAL FRONTAL LOBE lesions, 2 had lesions which extended into basal forebrain. The neuroanatomical and neuropsychological analyses on 5 patients presented in verbal form in a modified, non-real-time base. The impaired social behavior of patients with bilateral orbital lesions, can be seen as a domain-specific amnesia in which defective activation of learned somatic states plays a key role (Damasio & Tranel, 1988). Our theory predicts impaired autonomic responses to nonverbal "emotional/social" stimuli in these patients. We studied the effects of two stimulus configurations in 5 patients and 8 controls, 4 of whom were with both target and nontarget stimuli. Three subjects in each group were primed with target stimuli (e.g., a face) and with nontarget stimuli (e.g., a house). The results showed that patients with bilateral orbital lesions had significantly lower skin conductance responses (SCRs) to target stimuli than controls. The impaired social behavior of patients with bilateral orbital lesions, can be seen as a domain-specific amnesia in which defective activation of learned somatic states plays a key role (Damasio & Tranel, 1988). Our theory predicts impaired autonomic responses to nonverbal "emotional/social" stimuli in these patients. We studied the effects of two stimulus configurations in 5 patients and 8 controls, 4 of whom were with both target and nontarget stimuli. Three subjects in each group were primed with target stimuli (e.g., a face) and with nontarget stimuli (e.g., a house). The results showed that patients with bilateral orbital lesions had significantly lower skin conductance responses (SCRs) to target stimuli than controls.
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516.3

DOMAIN-SPECIFIC AMNESIA FOR SOCIAL KNOWLEDGE
A.R. Damasio, D. Tranel, Division of Behavioral Neurology & Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, Iowa.

Patients with bilateral lesions in orbital and lower mesial frontal cortices develop changes in social behavior that include inadequate decision making, planning, and conduct. These patients are unaware of their predicaments. The primary cognitive and neural mechanisms for these remarkable changes remain enigmatic and polemical. Based on extensive behavioral, neuropsychological and physiological experiments in 5 subjects, we propose that the deficits (1) stem from impaired consolidation of an appropriate range of memories relative to the complex activities, properties, and events that characterize social interactions, and (2) are manifest when "social knowledge" configurations are presented in real-time through neural channels. Prominent among the missing memories are somatic states, including those regulated by the autonomic nervous system. The new theory postulates that the reactivation of internal neural structures and interconnecting pathways which are necessary for the learning and retention of new experiences. These neural units include the medial temporal region, anterior medial diencephalon, basal forebrain, retrosplenial cortex and interconnecting pathways such as the fornix, mammillo-thalamic tract and amygdalolugalifugal pathway. Patients with damage to these and surrounding structures may also exhibit an extended retrograde amnesia beyond the 1-3 years prior to cerebral injury. We report here the results of neuroimaging in patient SS, who developed a severe anterograde and retrograde amnesia following four conditions lasting 60 s each: rest, visual learning, recall and recognition. The subjects learned ten colored geometric patterns which were contained in the same information than shape and color. The color contrast and luminance was balanced. During recall, the subjects, with eyes closed, recalled the appearance of each color-pattern in the same order as originally shown. During recognition the learned patterns were mixed with other similar patterns. Visual learning increased the rCBF in several prefrontal areas, orbital frontal cortex, insula, hippocampal regions, neostriatum, thalamus, the calcarine cortex, peri-calcarine cortex, and the visual association areas in the posterior superior parietal cortex and precuneus. Recognition of the color-patterns increased rCBF in the same structures. The purely intrinsic recall of the color-patterns did not change the rCBF in the calcarine, peri-calcarine and orbital cortex; the increase of rCBF in posterior thalamus was especially marked, apart from this the structures which increased rCBF were same as during learning and recognition.

516.5

ANATOMIC CORRELATES OF RETROGRADE AMNESIA, P.J. Eslinger and L. Damasio. Cognitive Neuroscience Lab, Brown University, Providence, Rhode Island 02903 and Memory Disorders Research Center, Boston VAMC, Boston, Massachusetts 02130.

Studies of patients with amnesia have identified a number of structures and interconnecting pathways which are necessary for the learning and retention of new experiences. These neural units include the medial temporal region, anterior medial diencephalon, basal forebrain, retrosplenial cortex and interconnecting pathways such as the fornix, mamillo-thalamic tract and amygdalolugalifugal pathway. Patients with damage to these and surrounding structures may also exhibit an extended retrograde amnesia beyond the 1-3 years prior to cerebral injury. We report here the results of neuroimaging in patient SS, who developed a severe anterograde and retrograde amnesia following encephalitis 17 years ago at the age of 38 (Neuropsychologia, Vol 21, 1983, 213-234). In addition to bilateral medial temporal lobe damage and possible damage to the posterior ventromedial frontal lobe (structures which have been associated with anterograde amnesia), SS sustained cortical damage to the insula bilaterally, to the temporal poles bilaterally, and to the left anterotemporal region. By comparison, patient DBB (Arch Neurol 42, 1985, 252-9) has a more severe retrograde amnesia and a highly similar but more extensive pattern of damage.

We conclude that in a neural model of memory, extended retrograde amnesia is related to damage in cortical structures of the insula and/or anterotemporal lobes.

516.6

A POSITION EMISSION TOMOGRAPHIC STUDY OF ANATOMICAL STRUCTURES IN THE HUMAN BRAIN PARTICIPATING IN LEARNING, RECALL AND RECOGNITION OF COLORED PATTERNS.
P.E. Roland, L. Kaiden and S. Stone, Dept of Clinical Neurophysiology, Karolinska Hospital and Institute S-104 01 Stockholm, Sweden.

The regional cerebral blood flow (rCBF) was measured with 11C-labelled OBM in ten young normal volunteers during four conditions lasting 60 s each: rest, visual learning, recall and recognition. The subjects learned ten colored geometric patterns which were contained in the same information than shape and color. The color contrast and luminance was balanced. During recall, the subjects, with eyes closed, recalled the appearance of each color-pattern in the same order as originally shown. During recognition the learned patterns were mixed with other similar patterns. Visual learning increased the rCBF in several prefrontal areas, orbital frontal cortex, insula, hippocampal regions, neostriatum, thalamus, the calcarine cortex, peri-calcarine cortex, and the visual association areas in the posterior superior parietal cortex and precuneus. Recognition of the color-patterns increased rCBF in the same structures. The purely intrinsic recall of the color-patterns did not change the rCBF in the calcarine, peri-calcarine and orbital cortex; the increase of rCBF in posterior thalamus was especially marked, apart from this the structures which increased rCBF were same as during learning and recognition.

516.7

MATERIAL-SPECIFIC MEMORY DEFECTS AFTER UNILATERAL TEMPORAL NEOCORTICECTOMY.
(Spon T. W. Parker & A.R. Dobbs* Dept. of Psychology, Camrose Lutheran College, Camrose, Alberta, T4V 2R3.

Two studies were carried out that examined aspects of memory in patients who had undergone a unilateral temporal neocorticectomy for the relief of intra-temporal neoplasms (Richet, Institute, Dublin, in Experiment I), or a compatible epileptic spell (Richet, Institute, Dublin, in Experiment II). In Experiment I), a forced-choice recognition-memory paradigm was used to assess memory for: (a) abstract words; (b) concrete words; (c) pictures of common objects; and (d) abstract geometric designs. The left temporal-lobe group (N=10) was impaired on the verbal recall test, the right temporal-lobe group (N=10) was impaired on the abstract designs. The results are consistent with those observed following temporal lobectomy. Experiment II examined the effect of eliminating the value of a verbal label on memory for pictures of common objects. The results revealed an impairment in the right-temporal-lobe group. The results of these two experiments extend, to the optimal level of temporal neocortical contribution to human memory processes.

Patient H.M., despite severe anterograde amnesia following bilateral medial temporal-lobe resection, has the learning capacity to substantially improve a mental rotation skill for mental rotation tasks (ERT, 1983). He and healthy, age-matched control subjects made verbal responses in judging whether a non-upright letter was in its normal or mirror-reversed form. The stimuli were viewed at each of 12 orientations, 30 degrees apart. On three successive days, subjects performed two sessions, an hour apart, each with 72 trials. As with control subjects, the initially moderate slope of H.M.'s reaction-time/rotation function declined to nearly zero, and his overall mean reaction time and errors decreased. These results suggest that declines of such RT function slopes are due to an increase in the rate of mentally rotating the stimulus in upright and not due to memorizing its form at the tested orientations. Prior reports of preserved perceptual learning have been limited to stimuli viewed at one orientation, and analysis of the conditions producing the preserved learning reported here may reveal the relation between long-term memory structures and processes used to discriminate disoriented stimuli. Our findings are consistent with the view that there is a dissociation in global amnesia between skill learning and fact learning. In 1987, we reported that H.M. showed no such improvement when this task required a button press response, a result apparently due to his difficulties with a forced-choice motor response. Supported by grants MH24433 and RR00088.


We report new examples of preserved priming in the globally amnesic patient H.M., and present a theoretical interpretation of intact and impaired priming in amnesia and in Alzheimer's disease (AD). We propose that repetition priming on a visual task takes three different forms. (1) Pre-representational priming depends upon perceptual learning mechanisms that facilitate access to representations (letters, words, objects): such mechanisms are spared in amnesia but are impaired in mild AD. (2) Post-representational priming depends upon learning mechanisms that guide semantic and lexical access to representations in long-term memory; these mechanisms are spared in amnesia but are impaired in more severe AD. (3) Post-representational priming depends upon mechanisms that record the outcome of operations on a representation; such priming is impaired in amnesia and AD. The observed dissociations among the three forms of priming suggest that they have separable neural bases. Dissociations among forms of priming may reveal how distinct neural systems enhance the efficiency of access to representations irrespective of subsequent operations performed upon those representations.

SUBSTANCE P AND SOMATOSTATIN COEXIST WITHIN SENILE PLAQUES OF PATIENTS WITH ALZHEIMER'S DISEASE. J. M. Armstrong, M. Benjamin, D. Evans, and L. Romans, Dept. of Neurosci., University of California, San Diego, La Jolla, CA 92039.

In recent years we and others have identified several neuropeptide and neurotransmitter-related substances within senile plaques of patients with Alzheimer's disease. At present, it is unclear whether a senile plaque can be identified according to a single transmitter or peptide substance or alternatively whether a plaque contains multiple substances. In the present study we employed a highly sensitive dual-immunolabeling procedure and demonstrated that substance-imunoreactive profiles exist within senile plaques of patients with Alzheimer's disease. Coexistence of somatostatin and substance P within senile plaques was observed in the hippocampus and amygdala but not in the neocortex, although the latter region contained plaques within which somatostatin and substance P existed alone. Regardless of the region, the labeled processes were usually enlarged and within the peripheral (i.e., the neuronal) portion of the plaque. Supported by NIH grants AG05344 and AG05386.

GLUCOSE-6-PHOSPHATASE ACTIVITY IN HIPPOCAMPAL NEURONS. K. Cullen-Dockstader and E. Ellisow (GPON, H. Alpert, Dept. of Psychology, University of Colorado, Boulder, Colorado 80309). Glucose-6-phosphatase (G6Pase) is intimately involved in the mechanism of Ca++ transport into the endoplasmic reticulum (ER). G6Pase activity in the ER hydrolyzes the glucose-6-phosphate (G6P) whereby it liberates phosphate ions. These are used to capture the actively transported Ca++ ions. These experiments have shown G6Pase activity in the smooth and rough ER and in the nuclear envelope. Basket cells, the inhibitory interneurons of the dentate fascia and hippocampus, show a considerably higher activity in the dentate granule cells or hippocampal neurons. Also, neurons of the hilus are intensely labeled. This method is an indicator of differential metabolic activities, and it shows that the Ca++ transport activity in the G6Pase activity associated with senescence. The ultimate goal of these experiments is to demonstrate the extent to which G6Pase activity may be affected by aging and thus the G6Pase activity in aged rats. Supported by the Institute on Aging, grant AG 04840-03.

Aging brings about a number of neuronal functions, out of which impairment of synaptic transmission is the most critical. Previously we have shown that migration of synaptic vesicles in the perforant path terminals towards the active synapse significantly decreased with age; however, no data are available about the size of synaptic vesicles in aged rats. In the present study, synaptic vesicles were found there at resting conditions. The obvious next step was to study the size (cross section) of synaptic vesicles in aged rats. Two groups of 3, 9, 24, and 30 months old were used. Five animals per age group were prepared for electron microscopy. Vesicles were measured in electron micrographs of the perforant path terminals in the dentate molecular layer. Perforant path terminals were identified as those contacting dendritic spines. The vesicle cross sectional area was computed from the largest diameter and the diameter perpendicular to it, using an IBM PC-based three-dimensional reconstruction program (Kinnaman et al., Proc. 44th EMSA Meeting, 1986, p 876). In these preliminary experiments, we have measured 250 terminals per age group. A significant decrease of the vesicle cross sectional area was observed by the 24th month as compared to the 3rd and 9th month. Since the majority of synaptic vesicles is generated by recycling, the present data could be interpreted as a failure of this process during aging. Such a change could bring about an impairment of the synaptic transmission, the severity of which would be greater with increasing age.

Supported by National Institute on Aging grant AG04804-03.


Studies on the aging hippocampal formation have revealed ultrastructural and physiological alterations that may underlie a number of functional deficits associated with aging. One such change is the reduced density of nuclear pores complexes (NPCs) in the granule cells of the dentate fascia which occurred in the absence of any changes in the nuclear envelope perimeter (Fikova et al., Exp. Neurol. 95, 1987). This decrease is thought to reflect a decrease in the metabolic activity of these cells. We report here our preliminary findings on the density of these pores in a different area of the hippocampal formation, the CA1 pyramidal cells. Ten male Fischer 344 rats aged 3, 9, 24, and 30 months (2 animals per age) were prepared for electron microscopy and were photographed on a JEM-100C electron microscope. There were no differences in the density of pores across ages in the CA1 pyramidal cells. There was, however, a 17% increase in the nuclear envelope perimeter with increasing age (which did not reach significance). Such an increase may have masked any change in NPC density. These data suggest that there may be age-related regional differences within the one structure for the same parameter. Moreover, more animals are required before definitive statements can be made regarding the effects of age on NPC density in this area.

Supported by NIA AG04804-03.


Low (CANP I) and high (CANP II)-calcium-requiring neutral proteinases (calpains) and their specific endogenous inhibitor (calpastatin) constitute an intracellular regulatory system that plays a major role in the metabolism of cytoskeletal proteins. Differences between the CANP activities in neonatal and adult brain regions have been reported; however, no data are available about their regulation and activities in the hippocampus. We measured CANP activities in six brain regions (cortex, cerebellum, striatum, pons-medulla, hypothalamus, and hippocampus) in young adult (1-month-old) rats and aged (24-month-old) rats, using 14C-methylated casein and brain protein substrates (desmin, actin, tubulin, and neurofilament). Activities of CANPs (high and low) were measured from the two enzyme fractions by Reaction Red 120-agarose. We could not detect significant CANP activity in any of the regions, only slightly in the pons-medulla and the cerebellum, with casein as substrate. In aging brain CANP II activity increased greatly in the cerebellum, only slightly in cortex and pons-medulla. Enzyme property alterations are indicated by the substrate dependence of regional activities changing with age. The determination of regional inhibitor levels is in progress to clarify its role in the age-dependent alterations of CANP activity. (Supported by NIH AG05670).

517.8 LEUPEPTIN CAUSES AN ACCUMULATION OF PHOSPHORYLATED TAU AND UBIQUITIN IN RAT BRAIN. G. Ochi, K. Kojima, and Y. Inagawa. Life Sciences, Univ. of Tokyo, Tokyo, Japan. (Supported by Naito Foundation.)

In Alzheimers disease (AD) and to some extent in aging, neurofibrillary tangles (NFT) are often observed. The formation of paired helical filaments (PHF) accumulate. Several laboratories have shown that the major antigenic components of PHF are phosphorylated tau proteins. It has been suggested that ubiquitin is present in NFT, possibly attached to phosphorylated tau itself. As an attempt at understanding the cellular mechanism underlying NFT formation, we administered either the protease inhibitor, leupeptin or buffered saline intravenicularly to rats for two weeks using an osmotic mini pump. Brain tissue from these and non-aged rats was processed for immunocytochemistry using antibodies to PHF (affinity purified to react with phosphorylated tau) and ubiquitin. Many Purkinje cells in the cerebellum of leupeptin treated and aged, but not saline treated rats, displayed increased immunoactivity to both antibodies. Anti-PHF antibodies labeled the perikarya and proximal dendrites while anti-ubiquitin stained perikarya, nuclei and portions of Purkinje cell dendrites. The finding that inhibition of thiol and some serine proteinases causes a buildup of abnormal neuronal inclusions with antigenic similarities to NFT and PHF supports the protease inhibitor hypothesis of its role in the mechanism of aging and AD (Ivy, 1987). Supported by NIA and NSERC.
LEUPEPTIN CAUSES SOME MANIFESTATIONS OF AGING IN THE RETINA
G. M. Smith and G. O. Ivy. Life Sciences, Univ. of Toronto, Toronto, Ont. M1C 1A4.

An accumulation of lipofuscin in the retinal pigment epithelium (RPE) is a hallmark of aging. This buildup may be due to a reduced ability of aged RPE cells to catabolize the contents of both phagosomes (mainly photoreceptor outer segment discs, PD) and autosomes. In this study, two month old Sprague Dawley rats were injected intravitreally, every 24 hours for several days; one eye with the protease inhibitor leupeptin (200 mg/ml) and the other with the same volume of saline. Eyes from young and aged rats were also examined. RPE cells of eyes from aged and leupeptin treated rats contained more Periodic Acid Schiff positive deposits compared to saline treated eyes. Electron microscopic analysis confirmed the presence of numerous dense bodies in the RPE of leupeptin treated as compared to saline treated eyes; the majority of these appeared to be composed of PD at various stages of catabolism, as seen in untreated eyes from young rats. Other deposits were displayed morphologically typical of lipofuscin seen in the aged rats. Further, varying degrees of photoreceptor degeneration were evident in many of the leupeptin treated and aged areas. Together, the results indicate that lipofuscin accumulation in RPE cells may be largely due to a buildup of PD caused by decreased proteolytic activity and that photoreceptor degeneration can be caused by protease inhibition. Supported by NIA and NSERC.

EFFECT OF AGE AND LONG-TERM OVARIETRY ON HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS ASSOCIATED WITH THE ESTRADIOL-STIMULATED LH SURGE. K. Sarabtshaw and P. M. Wise. Dept. Physiology, School of Medicine, Univ. of Maryland, Baltimore, Md. 21201.

E2-induced E2-secretion is age dependent in middle-aged compared to young rats. This age-related change in the pattern of LH release is associated with alternations in catecholamine turnover rates in hypothalamic nuclei known to be important in the regulation of the surge. Middle-aged long-term ovarioctomized (OVX) rats (OVX at 3 months) exhibit E2-induced LH surges which are similar in timing and amplitude to the surges displayed by young rats. We found that hypothalamic E2 surge activity is delayed in the rate constants of NE activity in microdissected hypothalamic nuclei.

BASAL GANGLIA/CEREBELLAR CALCIFICATION IN DOWN'S SYNDROME BRAINS. C. E. Hoebel*, J. D. Stedronsky*, J. A. L. S. Cohn*, and V. A. Armbrustmacher*. (SPON: E.Yeterian). Dept. Neurology, Children's Hospital, Boston, MA 02115, and Departments of Anatomy and Neurology, University of Washington, Seattle, WA 98195.

Cerebral and basal ganglia/cerebellar calcification/mineralization deposits were examined in 8 brains of the Yakovlev Collection, and 9 brains from Down's Syndrome (DS) individuals, 2.5 to 56 years old. Four brains (2.5, 4, 16 and 29 years) had no deposits, or trace amounts. The others (6, 26, 47 and 56 years) had deposits increasing in severity with increasing age. The oldest had deposits in the cerebellum also. The distribution, severity and local neuronal effects of basal ganglia/cerebellar calcification/mineralization deposits were examined in 6 brains of the Yakovlev Collection from Down's Syndrome (DS) individuals, 2.5 to 56 years old. Four brains (2.5, 4, 16 and 29 years) had no deposits, or trace amounts. The others (6, 26, 47 and 56 years) had deposits increasing in severity with increasing age. The oldest had deposits in the cerebellum also.

The calcification/mineralization deposits were extraneuronal and often appeared as rows of spheroids organized along blood vessels. Heavier deposits appeared as disorganized, conglomerates of spheroids. The site of greatest concentration in DS brains was in globus pallidus. In controls trace deposits when found were located both in globus pallidus and/or caudate/putamen.

Planimetric measures of cell body and nucleus cross-sectional area, and measures of cell packing density revealed no differences among affected and non-affected DS brains or age-matched controls. Neurons were clearly absent only in the area of heaviest deposit (bilaterally) in the most severely involved brain, and only in portions of the neuropil which had an abnormal, gelatinous appearance.


Department of Physiology, University of Illinois, Urbana, IL, 61801.

In the present experiment, 0.8 were dissected out of young (less than 100 days of age) and aged (greater than 500 days of age) rats. Homogenized O.B. tissue extracts were analyzed by radioimmunoassay to determine the basal levels of cAMP production. The tissues were also exposed to a submaximal concentration of an adenosine A1 agonist, which increased the amount of cAMP produced. The basal levels of cAMP production were increased in aged compared to young rats.
with Federal and Society guidelines). Changes in MAO activity were evident in some areas related to neuroendocrine function during aging are most evident in brain areas of young and old (3.5 and 25 months) Fisher (FCtx), striatum (St), suprachiasmatic nucleus (SCN), bed nucleus (AH, DMN and VMN). Comparisons between all aged and young rats display no alterations in morphologic or cell survival. However, cells plated 12 days earlier showed marked dendritic degeneration and neuronal swelling while those plated 14 days earlier had widespread neuronal loss. Increasing MAO activity during aging are most evident in brain areas of young and old rats, and the enzyme levels did not correlate with the degree of behavioral impairments in the aged rats.

The present results provide evidence that all major components of the forebrain cholinergic system undergo degenerative changes with age, and that these atrophic changes are correlated with the decline in spatial learning and memory in the aged rat.

GLUTAMATE NEUROTOXICITY DURING DEVELOPMENT & AGING. C. Peterson, J. Neal and C. Cotman Dept. Psychobiol., Univ. of CA at Irvine and Dept. of Neurol. Surg., USC Sch. Med., Los Angeles. Glutamate excitotoxicity has been implicated as an important factor in hypoxic-ischemic neuronal death. The resistance of young animals to hypoxia is correlated with the development of glutamate binding to NMDA receptors. The development of glutamate binding correlates to NMDA neurotoxicity, an in vitro neuronal preparation was used. Hippocampal neuronal cultures were prepared from rodent embryos (18-20 days). At certain days in culture, cultures were incubated with various concentrations of NMDA (50, 50, 0.5, 0.05 and 0.05 μM) for 5 min and alterations in cellular morphology and cell viability were noted 24 hr later. Cultures which had been plated 5 to 9 days earlier displayed no alterations in morphology or cell survival. However, cells plated 12 days earlier showed marked dendritomal swelling while those plated 14 days earlier had widespread neuronal loss. Increasing NMDA concentrations leads to a decline in cell survival. This finding suggests that neurotoxicity due to NMDA receptor activation can be developed in vitro.

Thus, the degeneration of hippocampal neurons in culture after acute NMDA treatment is age-dependent. This in vitro vulnerability correlates with the in vivo development of glutamate binding to NMDA receptors. The mechanisms of excitotoxicity in neuronal cultures may help develop potential strategies to prevent or reverse glutamate-induced neuronal degeneration. Supported in part by AG538, ADRDA and the French Foundation.
518.1


Our studies investigated the early developmental regulation of Na,K-ATPase activity in chick embryos. Na,K-ATPase activity increases rapidly between 11 and 18 day old rat brains in vivo. The percent radioactivity incorporated into individual lipids was also determined. Plasma levels of 3-HOB decreased from 2.24 to 0.36 and 1.38 to 0.41 mM in 11 and 18 day old rats in vivo.

518.2


PC12 pheochromocytoma is a subclonal line of PC12, which is a specific cofactor (electron donor) for aromatic amino acid hydroxylases, and found that BPH4 level was a substrate of GTP-cyclohydrolase which catalyzes the formation of 6-propyl-5-hydroxyindole (BPH4). The first step of BPH4 biotransformation into 6-hydroxy-BPH4 occurs in the fetal rat heart during development. In atrium, the highest levels of PAM mRNA were found at embryonic days 18-20 (E18-20); the levels declined rapidly at birth and increased transiently to the high levels found in adult atrium by postnatal day 14 (P14). In ventricle, the highest levels of PAM mRNA were found at E18-20, the levels diminished at birth, transiently increased until P7 and then declined to the low levels seen in adult ventricle by P14-P21. Both 3.6 and 3.8 kb forms of PAM mRNA were observed in atrium and ventricle, although their ratio varied significantly during the days studied. For atrium and ventricle, a soluble form of PAM activity was dominant from EL8 to adult.

518.3


518.4


518.5


Peptidylglycine α-amidating monooxygenase (PAM; EC 1.14.17.3) catalyzes the amidation of bioactive peptides. The PAM precursor predicted from the sequence of a cDNA cloned from bovine neurointermediate pituitary was a 108 kDa protein containing a hydrophobic putative membrane anchor domain near its C-terminus. High levels of membrane-associated PAM activity and PAM mRNA have been identified in atrium and ventricle of rat heart atrial and ventricular membrane preparations. The highest levels of PAM mRNA were found at embryonic days 18-20 (E18-20); the levels declined rapidly at birth and increased transiently to the high levels found in adult atrium by postnatal day 14 (P14). In ventricle, the highest levels of PAM mRNA were found at E18-20, the levels diminished at birth, transiently increased until P7 and then declined to the low levels seen in adult ventricle by P14-P21. Both 3.6 and 3.8 kb forms of PAM mRNA were observed in atrium and ventricle, although their ratio varied significantly during the days studied. For atrium and ventricle, a soluble form of PAM activity was dominant from EL8 to adult.

518.6


518.7

PC12 pheochromocytoma is a subclonal line of PC12, which is a specific cofactor (electron donor) for aromatic amino acid hydroxylases, and found that BPH4 level was a substrate of GTP-cyclohydrolase which catalyzes the formation of 6-propyl-5-hydroxyindole (BPH4). The first step of BPH4 biotransformation into 6-hydroxy-BPH4 occurs in the fetal rat heart during development. In atrium, the highest levels of PAM mRNA were found at embryonic days 18-20 (E18-20); the levels declined rapidly at birth and increased transiently to the high levels found in adult atrium by postnatal day 14 (P14). In ventricle, the highest levels of PAM mRNA were found at E18-20, the levels diminished at birth, transiently increased until P7 and then declined to the low levels seen in adult ventricle by P14-P21. Both 3.6 and 3.8 kb forms of PAM mRNA were observed in atrium and ventricle, although their ratio varied significantly during the days studied. For atrium and ventricle, a soluble form of PAM activity was dominant from EL8 to adult.
The distribution of GAP-43 in the monkey brain. K. Ando, M. Ohkuma, K. Tanaka, K. Hama, H. Hattori. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY and Dept. of Pathology, Ohio State, Columbus, OH.

GAP-43 is a neuron-specific phosphoprotein associated with the development, regeneration, and modulation of synaptic relationships. Using immunohistochemical techniques, the distribution of this protein was studied in the brains of adult rhesus monkeys.

Within the subcortical telencephalon, GAP-43-like immuno-reactivity was present in the ventromedial striatum, the olfactory tubercle, the substantia innomina, the bed nucleus of the stria terminals, and portions of the amygdala. Particularly dense deenecapthic staining was observed in midline structures such as the periventricular n. the dorsomedial n. of the thalamus, the parietal n., and in midline hypothalamic nuclei. Melanin and brainstem nuclei with similar dense staining included portions of the superior colliculus, the substantia nigra pars reticulata, interpeduncular n. dorsal raphe, parabrachial n. of the solitary tract, and dorsal motor n. of the vagus. In addition portions of the neocortex and hippocampal formation showed dense staining.

The distribution of GAP-43 in the adult monkey brain to some extent parallels that reported in the adult rat (Benowitz, et al. J. Neurosci. 8: 88). Concentrations of the protein are high in areas regarded as having limbic or associative functions. However, unlike the rat several regions associated with motor and somatosensory functions also showed relatively dense GAP-43-like immunoreactivity. The more extensive pattern of GAP-43 staining in the primate compared to that observed in the rat may reflect an increase in the integration or plasticity of these nuclei.


During development there is a characteristic transient appearance of acetylcholinesterase in the primary sensory regions of cerebral cortex and dorsal thalamic nuclei. It is not known, however, whether unique molecular forms of acetylcholinesterase are transiently expressed in these regions. Using sucrose gradient ultracentrifugation we have determined the molecular forms of acetylcholinesterase at various stages of brain development, in regions that express transient and conserved enzyme activity.

Brain regions were homogenized in the presence and absence of 1% triton X-100 and the extracts analyzed on 5-20% sucrose gradients.

Brain regions were homogenized in the presence and absence of 1% triton X-100 and the extracts analyzed on 5-20% sucrose gradients.

Analysis of the transient acetylcholinesterase indicated that it also included 10 S and 4 S forms predominated in the soluble extracts, while the 10 S form predominated in the insoluble, detergent extracted tissue.

Development of CORTICOSTERONE AND GROWTH HORMONE RESPONSES TO VARIOUS STRESSFUL PROCEDURES IN NEONATAL RATS. C. Gorenstein. K.A. Gallardo* and R.T. Robertson. Depts. of Pharmacology and Anatomy and Neurobiology, University of California, Irvine, Irvine, CA 92717.

Cortisol levels were measured in 4-day-old rat pups placed in an incubator for 15 or 60 min following: 1) no treatment; 2) subcutaneous (sc) injection of 0.9% NaCl; 3) anterior pituitary extract; 4) posterior pituitary extract; 5) ice anesthetization; or 6) ether anesthesia.

Cortisol levels were elevated relative to non-treated controls 15 min after treatments except ice anesthetization. These levels remained elevated after 60 min in both controls and the ice anesthetization group. GI levels were significantly reduced at 15 min for all treatment groups except ice anesthetization.

There was no significant change in cortisol levels after ice anesthetization. Ether anesthesia may contribute to the reduction seen in the influence of conditions on mild stress on something during the neonatal to weanling age period.
EFFECTS OF PRENATAL TREATMENT WITH THE SEROTONIN AGONIST 5-METHOXYTRYPTAMINE ON SEROTONERGIC AND DOPAMINERGIC ACTIVITY
A. Shemer, E. Azmitia, Dept. Biology, and P. Whitaker-Azmitia.
NY University.

We have developed an in vivo model for manipulating serotonin (5-HT) activity during development (Shemer et al., 1988). Pregnant rats are exposed to the 5-HT agonist 5-methoxytryptamine (5-MT) which causes the offspring to show reduced 5-HT outgrowth and deficits in behavior. The Bmax of 5-HT receptors is also reduced as a result of the prenatal treatment. In order to test these receptor characteristics, we challenged the drug treated neonates pharmacologically. The drugs used were acute 5-MT (1 mg/kg) to assess serotoninergic sensitivity and apomorphine changes in the dopaminergic system. Drug treated neonates were tested in a photocellometer at 30 days of age when there were no observable differences in activity between treated and control animals. Significantly less activity was affected by both drugs, 5-MT inhibited activity levels, and apomorphine increased activity levels. The drug neonates were unaffected by either drug. These results indicate that receptors in both the serotoninergic and the dopaminergic systems were significantly less sensitive as a result of prenatal exposure to 5-MT.


RATIONAL FOR USING MATERNAL PLASMA GLUCOSE AND SCINTILATION COUNTS FOR DETERMINATION OF FETAL GLUCOSE UTILIZATION.
D.E. Kostreva and J. Wood.

The operational equation of the Sokoloff [14C] deoxyglucose method (J. Neurochem. 28:897-916, 1977) is comprised of four components one of which, an integral, is derived from each animal's plasma glucose and [14C] scintillation counts. Four pregnant rabbits were anesthetized, intubated, ventilated and their uterus was exposed. A connecting branch of the uterine vein was cannulated for sampling of venous blood from a single fetal-placental unit. The maternal femoral artery and vein were also cannulated for sampling. A bolus of [14C] deoxyglucose, 100 uCi/kg, was injected i.v. into the maternal circulation and the timed blood sampling procedure was initiated. The integrals were calculated for both the maternal arterial, and the fetal-placental plasma samples. The percent error in measuring glucose utilization using the maternal versus fetal plasma values was between 2 and 6% in three animals and 16% in the fourth. These differences were not statistically significant. Quantification of local fetal glucose utilization can therefore be made using the maternal plasma values.

Department of Developmental Psychology, Telephone Psych. Research, New York, NY 10032 and Laboratory of Cerebral Metabolism, State University of New York-Health Science Center at Brooklyn, Brooklyn, NY 11201.

Adverse neurobehavioral effects have been reported in infants prenatally exposed to cocaine. We have found that there are lasting changes in metabolism in several brain regions of the male offspring using the deoxyglucose method of Sokoloff et al. (1977) 3H-sulfide binding was quantified and correlated with regions showing altered gluconeogenic activity. (Supported by NIDA grant RO4DA18).


Quantitative autoradiographic studies require correction for variations in quenching of tritium emissions by different brain regions. We investigated changes in regional tritium quenching during the first three weeks of neonatal life. Conditions were established for labeling the brain of 3-day-old rats using [3H]2-deoxyglucose (2DG) in neonatal rats using i.p. injections. Quench correction was determined by measuring 2DG labelled brain tissue before and after either chloroform extraction (P.C., 1981, J. Neurosci. Res. 33:34) or ethanol extraction (P.C., 1986, J. Neurochem. 45:334). Image analysis used DUMAS from Drexel Univ.

Percent Tritium Quench by Region

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean Percent Quench</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>28.1</td>
</tr>
<tr>
<td>BLA</td>
<td>26.5</td>
</tr>
<tr>
<td>CP</td>
<td>77.3</td>
</tr>
<tr>
<td>IC</td>
<td>33.9</td>
</tr>
<tr>
<td>LG</td>
<td>42.1</td>
</tr>
<tr>
<td>CC</td>
<td>46.8</td>
</tr>
</tbody>
</table>

PC = parietal cortex; BLA = basolateral amygdaloid n.; CP = caudate-putamen; IC = internal capsule; LG = lateral geniculate nucleus; CC = caudatocortical area; CP = parietal cortex; LG = lateral geniculate nucleus.
519.1 THE SUPRACHIASMATIC NUCLEUS (SCN) ARE ESSENTIAL EFFECT OF LGN LESIONS ON PHOTOPERIODISM AND BODY WEIGHT IN SIBERIAN HAMSTERS. N.F. Ruby*, N. Ibuka*, B.M. Barnes*, J. Dark, and I. Zucker. Dept. of Psychology. Univ. of California, Berkeley, CA 94720.

Daily torpor was studied in hamsters, Phodopus sungorus, maintained in a short day photoperiod (8 hr light/16 hr dark) and implanted bilaterally with radiofrequency transmitters were transferred to a cold chamber (15°C) at 60 days of age. Body temperature (Tb) and locomotor activity data were collected at 10 minute intervals using a telemetry system and stored by computer for subsequent statistical analysis. Animals manifesting two torpor bouts (Tb<30°C) in one week for two consecutive weeks received lesions of the SCN, pinealocysts, or sham operations. In the 15 weeks following surgery none of the animals with bilateral SCN lesions showed torpor, circadian body temperature, or activity rhythms as determined by Fourier analysis. All pineolectomized hamsters continued to show torpor for at least 6 weeks postsurgically, as did the sham-operated groups.

Daily torpor, once initiated, persists in the absence of the pineal gland and melatonin. Expression of torpor and manifestation of circadian body temperature and activity rhythms require intact suprachiasmatic nuclei.

Supported by NICHHD Grant HD-02982.


The role of endogenous opioid in the trophic-dependent control of LH was investigated by comparing the effects of somatostatin, norepinephrine and melatonin on LH secretion in photostimulated (PS, 16L:8D), photoinhibited (PI, 8D:16L) and photorefractory (PR, 8D:16L) hamsters. Blood LH content was determined 4 hours after the drug administration.

In PS animals somatostatin or melatonin administration increased, and norepinephrine administered, serum LH. These manipulations were ineffective in PI animals maintained in SD for 12-18 weeks. Corticosteroid and drug responses were re-activated after 22 weeks in SD with the onset of LH (as determined by testicular regression). The LH response to corticosteroid stimulation response therefore varied in parallel with that of ontogenic manipulations.

Exp. 1. PS or PI hamsters (after 8 weeks SD) were castrated and either returned to the SD or transferred to a cold chamber (15°C) at 60 days of age. LH secretion was responsive to opiateergic manipulations.

Exp. 2. PS or PI hamsters (after 8 weeks SD) were castrated and either returned to the SD or transferred to a cold chamber (15°C) at 60 days of age. LH secretion was responsive to opiateergic manipulations.

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Supported by NIH Grant HD-02982.
SHORT DAYLENGTHS ALTER ELECTROPHYSIOLOGICAL RESPONSES OF HAMSTER SUBPOLEAR NUCLEUS (SONO) TO MELATONIN. B. Busak and R. Mason*, Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

Several experiments have suggested that the SCN is a target for melatonin effects that regulate seasonal reproductive cycles. We investigated whether melatonin alters firing rates of cells in the SCN of Syrian hamsters. Male hamsters were kept in a short photoperiod (10 h light daily for more than 150 days) to induce gonadal regression and spontaneous recrudescence. The reproductive systems of these hamsters become insensitive to exogenous melatonin. We compared the responses of their SCN neurons to those in animals housed in long light (14 h) or light (8 h), who are sensitive to melatonin. Extracellular recordings were made from hypothalamic slices in vitro to assess the effects of pressure ejection of melatonin (2 µM) on spontaneous firing rates. In long-day hamsters, melatonin evoked dose-dependent responses in approximately half the cells tested; unresponsive cells were encountered most frequently during the projected dark phase. About twice as many cells were suppressed as activated by melatonin. In contrast, nearly all SCN cells recorded in short-day hamsters were unresponsive to melatonin. These data suggest that functional insensitivity to melatonin may reflect in part a loss of neural responses to melatonin in the SCN.

Supported by NSERC and MRC of Canada, the Royal Society (UK), and Dalhousie RDFS.


The cholinergic agonist, carbachol, mimics the phase-shifting effects of light on (1) pineal NAT activity in rats and (2) the circadian rhythm of locomotor activity (CRLA) in mice and golden hamsters. These results suggest that the effects of light on the circadian system may be mediated by cholinergic mechanisms. The purpose of the present study was to determine whether the phase-shifting effects of light on the CRLA in Dungarian hamsters (Phodopus sungorus) can be mimicked by carbachol. The Dungarian hamster is unusual, because two species in which the phase advance region is limited to the light subjective night (SN), the phase response curve (PRC) to light in Dungarian hamsters has an additional small phase advance region in the late subjective day (SD). Blind adult male Dungarian hamsters received intracerebroventricular injections (i.c.v.) of either 10 nmol carbachol or vehicle at one of 12 circadian times, and PRCs were generated. Carbachol induced significant phase delays at CT 8 and 10 (p < .05). The PRCs for the CRLA in the hamster both have phase delay regions in the early SN and small phase advance regions in the late SD. Although the carbachol PRC does not appear to have a phase advance region in the late SN as does light, these results indicate that carbachol mimics some of the effects of light on the circadian system in Dungarian hamsters and support the hypothesis that the photic stimuli that light the circadian clock of mammals are mediated, at least in part, by cholinergic mechanisms.

Supported by NIH HL14388.


Age-related dysrhythmia may be the result of abnormalities in the SCN-based, light entrainable oscillator. Entainment to food restriction schedules (FR) appears to occur through an oscillator anatomically distinct from the SCN. The objective of the present study was to examine the response of the body temperature rhythms of intact, SCN-lesioned and aged rats to FR. Rats exposed to FR displayed a 14-h sleep-wake cycle; the lights were on for 14 days, with food available from 1300-1500. Following FR the animals were given food ad libitum for 3 days, and then food deprived for 2 days. Body temperature was measured by telemetry. During both FR and food deprivation, animals from all groups showed elevations in body temperature during the light phase and preceding food availability by 2-4 hours, a pattern resembling that of activity bouts displayed by rats exposed to FR. The food-entrainable oscillator appears to be intact in the aged, dysrhythmic rats. Support by NSF grant DCR-85-08187 and AFAR grant to BTO.
**FRIDAY AM**

**BIOLOGICAL RHYTHMS: SYSTEMS II**

**1299**

**519.13**

**CORRELATION OF SERUM CATECHOLAMINES WITH BEHAVIOR AS A FUNCTION OF PERIODIC TIME FACTORS AND TIME OF DAY**

D. B. Peeler and I. Harari*

University of Mississippi Medical Center, Jackson, MS 39216.

Serum epinephrine (E) and norepinephrine (NE) levels of male mice (n=12) from six inbred strains (C57BL/6, BALB/c and their 7 recombinant inbred strains) were determined for several times of day using HPLC with electrochemical detection. Activity levels for each strain at each time of day were compared with performance of these strains in previous studies of activity, emotion and learning.

Activity levels were significantly higher than those of morning serum samples. Afternoon serum typically had higher levels of NE and lower levels of E than did morning samples. Strain E and NE levels were significantly correlated for morning but not for afternoon samples. Levels of NE were significantly correlated with activity levels for the next 4 days, but not with other emotion measures - defecation, nor with activity. Morning NE correlated with investigatory activity at that time of day or later, while other measures were only correlated with other times of day or locomotor activity or emotion measure of E.

**519.14**

**RUNNING AND A MONOAMINE OXIDASE INHIBITOR ALTER SEROTONIN CIRCADIAN RHYTHM IN HAMSTER SUPRA-CHIASMIC NUCLEI IN DIFFERENT WAYS**

J. S. Kruse

Center for Brain Research & Psychology Department University of Rochester, Rochester, N.Y. 14642.

Serotonin (5HT) fibers in the suprachiasmatic nucleus (SCN) mediate delays in free-running and entrained running wheel circadian clock. Monamine oxidases (MAO) are the enzymes that metabolize monoamines in extraneuronal compartments. We have previously observed a decrease in Tb maximum. No significant effects were observed for 

**519.15**

**EFFECTS OF CHRONIC CLORGYLINE ON CIRCADIAN WHEEL RUNNING ACTIVITY IN NORMAL AND THYOPARATHYROIDECTOMIZED (TPX) RATS**

J. Schulz, W. Duncan, E. Bath*, D. Havercost*, and J. Walker*

Haverford College, Haverford, PA 19041.

Hypothalamic and pituitary glands are associated with thyroid abnormalities, shortened circadian periods of physiology and behavior, and elevated sensitivity to activity-suppressing effects of light. We now report that TPX males do not display abnormal rhythms to a single food pellet could last up to more than two weeks. The newly entrained rhythm displays peculiar circadian features:

- Increased correlations between the morning E level and the phase of the circadian cycle in which food is applied. The newly entrained rhythm displays peculiar circadian features:
- Afternoon NE levels correlated only with defecation scores obtained at the same time of day. Afternoon E levels correlated only with locomotor activity at that time of day.

**519.16**

**EFFECTS OF p-CHLOROPHENYLALANINE ON CIRCADIAN RHYTHMS OF BODY TEMPERATURE, DRINKING AND ACTIVITY**


Department of Psychology, University of Illinois, Champaign, IL 61820.

Serotonin (5HT) has been implicated in the control of thermoregulation. p-Chlorophenylalanine (pCPA) is a tryptophan antagonist that depletes brain levels of 5HT.

We examined the effects of pCPA on the circadian rhythms of body temperature (Tb), drinking and activity. pCPA administered to unrestrained and unhandled male and female Long-Evans rats (n=7-8/500 mg/kg i.p.) was monitored by telemetry telemetry 10 minutes, 10 minutes, and 50 minutes after the injection. A single pCPA injection (300 mg/kg i.p.) was given at 12:12 LD cycle and Tb minimum and the decreases in amplitude on day 2 through 3 are due to an increase in Tb minimum and a decrease in Tb maximum. No significant effects were observed after day 3.

pCPA decreases the nocturnality of drinking from 63±1% to 63±7% on day 2 (p<.05), and to 63±3% on day 3 (p<.01). No significant effects were observed after day 3.

Research supported by NIMH Grant #1RO1 MH 41138 to E.S.

**519.17**

**ENTRAINMENT BY FOOD OF CIRCADIAN LOCOMOTOR ACTIVITY**

J. J. Clavish, F. Fernandez de Miguel*, and W. Arechiga*

Dept. de Biologia y Neurociencias, Instituto de Fisiologia, Instituto y Neurociencias, Apartado Postal 14-740, Mexico, D.F. 07000, Mexico.

Animals were housed in computer-monitored running wheel cages. The experiment was conducted in three stages. After two weeks of Entrainment (LD 12:12), animals were implanted with either an advanced or a conventional chronotype. Results suggest that TPX males do not display abnormal rhythms to a single food pellet could last up to more than two weeks. The newly entrained rhythm displays peculiar circadian features:

- Increased correlations between the morning E level and the phase of the circadian cycle in which food is applied. The newly entrained rhythm displays peculiar circadian features:
- Afternoon NE levels correlated only with defecation scores obtained at the same time of day. Afternoon E levels correlated only with locomotor activity at that time of day.

**519.18**

**CIRCADIAN VARIATION IN PLASMA MHPG LEVELS**


Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106.

We have previously observed a decrease in Tb maximum. No significant effects were observed for 

**519.19**

**SEROTONIN CIRCADIAN RHYTHMS IN HAMSTER SUPRA-CHIASMIC NUCLEI**


Department of Psychology, University of Illinois, Champaign, IL 61820.

Serotonin (5HT) has been implicated in the control of thermoregulation. p-Chlorophenylalanine (pCPA) is a tryptophan antagonist that depletes brain levels of 5HT.

We examined the effects of pCPA on the circadian rhythms of body temperature (Tb), drinking and activity. pCPA administered to unrestrained and unhandled male and female Long-Evans rats (n=7-8/500 mg/kg i.p.) was monitored by telemetry telemetry 10 minutes, 10 minutes, and 50 minutes after the injection. A single pCPA injection (300 mg/kg i.p.) was given at 12:12 LD cycle and Tb minimum and the decreases in amplitude on day 2 through 3 are due to an increase in Tb minimum and a decrease in Tb maximum. No significant effects were observed after day 3.

pCPA decreases the nocturnality of drinking from 63±1% to 63±7% on day 2 (p<.05), and to 63±3% on day 3 (p<.01). No significant effects were observed after day 3.

Research supported by NIMH Grant #1RO1 MH 41138 to E.S.
519.19


We have developed a monkey model for studying circadian effects on performance. However, in many of the natural uses for this information -- jet-lag, shift work, and SP -- long-term influences on performance. Therefore, we conducted an experiment to explore the effects of SP on fatigue and its interaction with circadian rhythms. Adult rhesus monkeys were trained to asymptote on a vigilance-disinhibition (Vig-Diso) task and equipped to record circadian activity and temperature -- recorded at the beginning and before SP. During SP, successful performance was achieved by reinforcing with a food pellet 50% of the time, with trials every 2.4 min on average around-the-clock. This schedule produced continuous performance for at least 48 hr. Performance lapses ranging from 2-3 min appeared during day 3 of SP, and performance lapses up to 72 min were seen in day 4 of SP. Despite lapses, monkeys performed at least 85% of trials through hour 108 of SP. Performance degraded dramatically during SP, and SP was impaired during SP. Vig was impaired 51.9% (p < 0.001) and Vig at 64% (p < 0.001) during SP hours 72-84. Circadian performance and physiology rhythms continued through SP, though fatigue altered both. These results show that we can use SP to study dramatic fatigue effects on performance and rhythms in monkeys. Supported in part by VA Res & USAMRDC.

519.20


An improvement in mood after the spring adjustment of daylight saving time (DST) has been reported. There are as yet no published accounts of the effect of DST on psychiatric illness.

Three groups were studied with regard to DST. In each case the analysis compared the week prior to the week following DST. The presentation of paraesthesia to a regional pattern unit in Pittsburgh (population 500,000) was considered. Over the years 1976-1986 there was no consistent pattern with regard to the spring or autumn DST change.

Admissions to the only psychiatric hospital serving the same population over a 16 year period were studied. All diagnoses entered in the Edinburgh Case Register were classified as having a possible affective diagnosis or not. There was no influence of DST in either group.

Information of all suicide deaths in Scotland over the period 1974-1985 was made available by the Scottish Home Office. An analysis of the date of death in relation to DST did not reveal any pattern either in the male or female subgroups.

In none of the three populations studied was there a discernible influence of DST. It may be that certain individuals are susceptible to small changes in circadian rhythm but this has not been reflected in the groups of this study with varying psychiatric morbidity.

INFECTIOUS DISEASES

520.1


The sequential development of the behavioral effects due to CNS infection with the herpes simplex type I virus was examined in two monkey models. Following intracerebral infection of adult female VNA/Nylar with the HF strain of herpes or adult female Balb/c mice with the F strain the majority of animals survived. An increase in motor activity observed 7 days following infection of VNA/Nylar injected with the HF strain was measured by a standard plaque assay. Likewise an increase in evasive behavior occurring during serial reversal performance in a water F-maze was observed 8 days following infection of Balb/c mice and coincided with the declining phase of the viral growth curve. Taken together these results suggest that processes involved in the elimination of virus from brain, such as the cellular immune response, may be important in the development of the behavioral pathology produced by non-lethal herpes encephalitis.

520.2

EXAMINATION OF THE HSV INFECTION IN THE NEURON IN CULTURE C. C. Wilsa, R. B. Smith, F. E. Johnson, A. D. L. Price, and J. E. K. Shaw. Department of Microbiology and Immunology, and Pediatrics, University of Colorado Medical School, Denver, CO 80262, Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Herpes simplex virus (HSV) latency and viral gene expression was investigated in sympathetic neurons. Previously we showed that deprivation of nerve growth factor results in reactivation of latent HSV. We found that inhibition of protein synthesis, pharmacological agents which elevate cyclic AMP, activation of protein kinase C with phorbol ester, and heat shock produced reactivation of latent virus. HSV gene expression was examined during the productive infection, during latency and during the course of reactivation of latent virus in the neuronal cultures. Using conditions described previously which result in the establishment of latent HSV infections in neuronal cultures, viral gene expression was below the limits of detection by dot blot analysis immediately after inoculation with virus, and at subsequent time points during the latent infection. Preliminary results using in situ hybridizations suggest that viral gene expression is limited during latent infection, however the latency associated transcript was detected.


520.3

EFFECTS OF MUMPS VIRUS (MV) ON Na+ AND Ca++ CHANNELS OF PC12 AND TE671 CELLS. R. R. Stauffer and R. J. Ziegler. School of Medicine, Univ. Minn. Duluth, Duluth, MN 55812.

Persistent MV infection has been shown to reduce the excitability responsiveness of PC12 and TE671 cells (Zieglers and Stauffer, 1987, New Engl. J. Med.) and TE671 cells are altered by MV. Stimulated action potentials (SEAPs) were recorded intracellularly from mock-infected (MI) and MV-infected cell cultures. Standard ionic channel blockers (tetrodotoxin, tetroethylammonium ion, TEA; and cobalt ion, Co++) were used to differentiate and identify SEAPs typical of Ca++ spikes for PC12 cells remained. In all three cases, treatment with Co++ abolished the persistent SEAPs.

The results indicate that MV is exerting its effect via voltage-gated Na+ channels. Ca++ channels appear not to be influenced by MV as any appreciable extent. Supported in part by the Minnesota Medical Foundation and the Duluth Clinic Education and Research Foundation.

520.4


HIV has been shown to infect the nervous system, and, in brain, has been associated with cognitive and behavioral changes which can be independent of the clinical manifestations of other AIDS-related disorders. Patients at various stages of illness with serologic evidence of HIV infection and without signs of gross or focal neurological disorders were recruited for study. The subjects underwent comprehensive cognitive evaluations in order to characterize the nature of the intellectual decline associated with HIV infection.

A distinctive and consistent pattern of deficits was demonstrated, including impairments of attentional, organizational, and problem-solving abilities. Other abilities such as language, visual-spatial perception, and memory consolidation were relatively preserved. The deficits suggest compromise of a particular cerebral system composed of subcortical activating centers and projections to prefrontal cortex. This is consistent with evidence from neuropathology and neuroimaging studies indicating a predilection of HIV in brain for subcortical structures and white matter.

By use of double label histochemistry it has been possible to demonstrate that the neurosecretory cells of the adrenal medulla can be infected with Cytoivirus in the AIDS patient. While infection can eventually lead to cell death, it is important to know if the quantity of biologically relevant peptides that the adrenal medulla products can be altered during the course of infection. In order to test this possibility, a new double label method was developed. The PAR method was used to identify virus, while cell products (dopamine, chromogranin, synaptophysin, etc) were semi-quantified by radioligandimmunochemistry. This was done by use of a 125I-streptaviden label (Amersham). It was then possible to compare grains between uninfected and infected cells. Toxins and other agents can alter cell products.潘多玛尼已被发现是链状的。一个案例是被放射性标记的，通过半定量的手段在中性侵入产物产生。此方法得到证实，通过双标记免疫组织化学方法，发现被感染的细胞和未被感染的细胞之间的免疫染色强度有显著差异。被感染的细胞中的免疫染色强度比未被感染的细胞中的免疫染色强度高。被感染的细胞中，免疫染色的强度的变化可能反映了细胞的活力，当细胞没有活力时，免疫染色的强度会降低。因此，这个方法可以用来评估细胞的活力变化。
521.3 COMPENSATION FOR MUSCLE YIELD AND LACK OF STIFFNESS REGULATION BY STRETCH REFLEXES. R.R. Carter, P.E. Graup and M.M. Keith. Dept. of Biomedical Engineering and Orthopaedics, Case Western Reserve University, Cleveland, OH 44106.

The functional contribution of the Golgi Tendon Organs (GTO) in movement has been tested mainly in animal lower limb muscles. This study investigated the hypothesis that the facilitatory changes observed with controlled hip flexion in our previous studies were the results of affected central regulation from stretch of the hamstrings group was investigated.

Soleus H-reflex recruitment curves were generated in 7 healthy subjects at one control (15° hip flexion) and two test positions: (1) hip flexion (40°) and (2) hip flexion (40°) plus knee flexion (90°). H-reflex testing followed a standard procedure (15 msec pulse width, 0.1 Hz). In position 1, Hmax increased in 3 subjects (10-20%), decreased in 1 (11%) and was unchanged in 3. In position 2, 2 subjects showed a marked decrease in Hmax (-15 to -30%) and 1 showed an increase (18%). Only 1 of the subjects showing an increase in position 1 showed a decrease in position 2. The latter finding is not strongly supportive of the idea that hamstrings stretch contributed to the facilitatory effects of hip flexion alone on the soleus H-reflex. The pronounced inhibition seen in position 2 may be attributable to additional inhibitory inputs associated with the knee flexed position.

521.4 MODULATION OF SOLLEIS H-REFLEX EXCITABILITY WITH CHANGES IN HIP AND KNEE POSITION IN MAN. B.J. Sullivan, J. Pompera and C.E. Chapman. Centre de recherche, Institut de Readaptation de Montréal; Concordia University; Université de Montréal, Montréal, CANADA.

The hypothesis that the facilitatory changes observed with controlled hip flexion in our previous studies were the results of affected central regulation from stretch of the hamstrings group was investigated.

Soleus H-reflex recruitment curves were generated in 7 healthy subjects at one control (15° hip flexion) and two test positions: (1) hip flexion (40°) and (2) hip flexion (40°) plus knee flexion (90°). H-reflex testing followed a standard procedure (15 msec pulse width, 0.1 Hz). In position 1, Hmax increased in 3 subjects (10-20%), decreased in 1 (11%) and was unchanged in 3. In position 2, 2 subjects showed a marked decrease in Hmax (-15 to -30%) and 1 showed an increase (18%). Only 1 of the subjects showing an increase in position 1 showed a decrease in position 2. The latter finding is not strongly supportive of the idea that hamstrings stretch contributed to the facilitatory effects of hip flexion alone on the soleus H-reflex. The pronounced inhibition seen in position 2 may be attributable to additional inhibitory inputs associated with the knee flexed position.

It has been shown that the "late" (R III) component of a cutaneous reflex may be elicited by innocuous stimuli, whereas the "late" (R III) component requires painful stimuli (Everett, 1979). However, our preliminary experiments suggested that non-painful stimuli might produce both components. The present study focused upon this question. Subjects were relaxed, normal adults. Surface EMGs were recorded via electrodes positioned over the posterior or peroneus longus muscle. Stimulus presentations approach one min. Notably, previous failures to observe this SRII component have occurred in studies which utilized much briefer interscan intervals (3-5 s) but which were comparable to ours in other respects. Supported by NSF Grants BNS 8415917 and BNS 8808495.

521.10 EXTEROCEPTIVE REACTIONS IN MAN: STARTLING RESPONSES TO SHOCKING EXPERIENCES TO ORGANIZED RESPONSES IN HUMAN ARM MUSCLES. D. G. Kukulka, T. J. Cook

The present study was designed to survey the excitatory EMG reflex activity induced by the presentation of non-painful cutaneous or auditory (click) stimuli. Subjects were normal, relaxed humans in a supine position. Single clicks were delivered through padded earphones. Electrical stimuli, of length of train of pulses, were delivered once each minute to various skin sites. Surface EMG recordings were obtained from electrodes overlaying each of several different muscles.

Stimulation could induce both short and long latency reflex responses in muscles located relatively near the site of stimulation. In contrast, similar stimulation tended to trigger only the longer latency responses in muscles remote from the stimulation site. These findings are consistent with the following working hypothesis: "Earliest" reflex components have a local segmental organization, while "late" components result from supraspinal activation organized in a pattern reminiscent of the "startle reflex" (e.g., Landis and Hunt, 1939). Supported by NSF Grants BNS 8415917 and BNS 8808495.

522.1 THE ACTIVATION OF MOTOR UNITS IN REFLEX-INDUCED AND VOLUNTARY CONTRACTIONS IN HUMAN ARM MUSCLES. D. Brown, W. van Dongen, E. van Doylem, J. Denier van der Donck, Dept. of Medical and Physical, Univ. of Utrecht, The Netherlands.

Motor-unit activity in human arm muscles was recorded with intramuscular electrodes during isometric contractions and during reflex activity elicited by torque perturbations in the elbow during isometric elbow extension and elbow flexion. Short latency responses to loading and unloading perturbations were found only in muscles that were not stretched or shortened. These findings demonstrate that the medium latency reflex activity at medium latency were also observed in muscles that were not stretched or shortened. Therefore, the medium latency reflex cannot be the result of a simple feedback mechanism that controls muscle length only.

During medium-latency reflex activity different types of motor-units were found in nearly all muscles, each with a different type of activation. The relative activation of these subpopulations in the medium latency reflex for different directions can be explained by a combination of the temporary change in muscle stretch and muscle activity. Predictably, the activation of the medium latency reflexes is mediated by the same coordination center.

522.2 HUMAN FLEXOR REFLEX REVERSAL IN KNEE MUSCLES DURING CYCLING. D. Rabilloud, Z. Hunt, J. Hunt, R. B. Stein, The University of Iowa, Iowa City, IA 52242.

The human flexor reflex (HFR) in lower extremity muscles has been studied almost exclusively during static resting and static isometric conditions. In an attempt to characterize this reflex during a locomotor movement, subjects cycled at a constant workload while an electrical stimulus was delivered to the tibial nerve at the medial malleolus (0.1 ms rectangular pulses at 300/s for 25 ms). EMG responses recorded in tibialis anterior (TA) and biceps femoris (BF) at various phases of knee motion were compared to the mean EMG activity occurring at these intervals with no stimulus present. Results indicate a pattern of inhibition-excitation-inhibition in BF during knee flexion. The peak amplitude of the excitatory responses are dependent on both the mean baseline EMG activity and the knee angle at which stimulation occurs. The observed patterns strongly support previous studies investigating phase dependent reflex reversal in animal and human subjects.

Supported in part by NYS Grant #8259102

522.3 EVIDENCE FOR PHASE DEPENDENT CUTANEOUS REFLEX REVERSAL DURING WALKING IN HUMANS. J. F. Yang, R. B. Stein, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2N7.

Phase dependent cutaneous reflexes in walking have been reported for some time in cats, but never demonstrated in humans. This study examines these responses in human subjects. Fixation in flexion/extension, supination/pronation position. Short latency responses to loading and unloading perturbations were found only in muscles that were not stretched or shortened. These findings demonstrate that the medium latency reflex activity at medium latency were also observed in muscles that were not stretched or shortened. Therefore, the medium latency reflex cannot be the result of a simple feedback mechanism that controls muscle length only.

During medium-latency reflex activity different types of motor-units were found in nearly all muscles, each with a different type of activation. The relative activation of these subpopulations in the medium latency reflex for different directions can be explained by a combination of the temporary change in muscle stretch and muscle activity. Predictably, the activation of the medium latency reflexes is mediated by the same coordination center.

522.4 CONSTANT ERRORS IN HUMAN FORCE ARE PREDICTED BY CONTRACTION-INDUCED PLASTICITIES IN THE STRETCH REFLEX. A. S. Hutton, R. C. Power, H. K. Kelsh, and S. Suzuki. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Conditioning against muscle contractions cause post-contraction afterdischarge and increased stretch sensitivity in muscle spindle receptors. Under static conditions the tonic stretch reflex is enhanced leading to nonvolitional increments in muscle activity. Predictably, when human subjects estimate a previously learned criterion force (LCF) following a conditioning contraction, estimates of the LCF are overestimated compared to control values. The amplitude of these constant errors decreases over time as the intensity of muscle spindle afterdischarge, and both are attenuated by muscle stretch (Hutton et al., J. Physiol., 393, 247-259, 1987). Since afferents synapse with Ia reciprocal inhibitory interneurons, a maximum elbow extensor force (5 s duration) was performed followed by an FLEXOR ICF (25%) should induce underestimates in force due to afterdischarge-linked reciprocal inhibition from the extensors. This prediction was tested in 25 humans. Following an extensor conditioning force, estimates of the flexor LCF were consistently underestimated. The size of the underestimate was time dependent. Previous findings of an overestimate of a flexor LCF following a maximum flexor contraction were replicated. The direction of constant errors in humans can therefore be explained by known contraction-induced plasticities in muscle spindles.

Supported by MRC and AHRMR.

Vibration stimuli (1, 2, 3, or 9 cycles; 0.7–1.3-mm amplitude, 120Hz) were applied to reduce e.m.g. activity commencing at about 45ms, lasting some 20ms and reaching a minimum of approximately 55% of the pre-stimulus level. In about 25% of trials a shorter latency (10ms), smaller (minimum of 70% pre-existing level) depression of e.m.g. was present. The amplitudes of both phases of inhibition increased in proportion to background e.m.g. for voluntary ECR contractions of 10–30% maximum. Neither phase was appreciably affected by changes in stimulus duration. Comparable patterns of reciprocal inhibition of FCR were observed, in 6 subjects, upon vibration of the belly of ECR.

Out findings, in the wrist musculature, of short-latency, reciprocal inhibition, possibly mediated by Gq iso, oligo-synaptic, spinal reflex arc, and later, more prolonged reduction in motor discharge agree with earlier descriptions from h-reflex studies (Cavallari, et al., Exp. Brain Res. 56, 574, 1984; Day, B.L. et al., J. Physiol. 349, 515, 1984). We have observed that reducing (via true anesthesia) neural depression of e.m.g. was present. The amplitudes of both phases of inhibition increased in proportion to background e.m.g. for voluntary ECR contractions of 10–30% maximum. Neither phase was appreciably affected by changes in stimulus duration. Comparable patterns of reciprocal inhibition of FCR were observed, in 6 subjects, upon vibration of the belly of ECR.


Intramuscular injection of 1.0 Novocain (4–10cc) was used to block the stretch reflex of vastus lateralis (VL) to investigate stretch reflex contribution to stretch–shortening contractions with either tonic or dynamic pre-stretch. Six male athletes performed squat–jump relaxed (SJ, from seated on a chair), squat–jump tensioned (SJT, from knee flex to straightened), squat–jump hop (SJH, one leg), counter–movement jump (CMJ, with fast pre-stretch), counter–movement hop (CMH, on one leg), and drop jump (DJ, from 50 cm high box) trials before and after injection. Ground reaction forces were measured; EMG from VL was rectified and integrated (EMG). Ten IEMG responses to tendon tap were averaged before and after injection. Reflex amplitude decreased 52–87%. Repeated measure ANOVA was used to analyze JVM height (H), maximum force, IEMG, and vertical velocity (V) with no change occurring in SJR variables. Muscular function without pre-stretch was not compromised. Significant decreases occurred in both tonic / H = SJH(75); V = SJH(50) / and dynamic / H = CMJ(150), DJ(105); V = CMH (71); IEMG = CMJ(205), CMH(255) / pre-stretch conditions. Two leg trials may include some contralateral leg compensation. The stretch reflex contributes more to enhanced muscle function during dynamic pre-stretch than tonic pre-stretch.


Patellar tendon tap response to a maximum tap force were compared in an able-bodied group (AB,n=20) and a spastic group (SP, n=10). In both groups, peak amplitude (AMP) of the largest spike, and muscle force (FORCE). SP subjects consisted of spinal cord injured with no voluntary movement for AMP (CPM, x=1478 uV, SCM, x=741 uV; CMH, x=264 uV), and FORCE (CPM, x = 43.4 N; SCM, x=38.0 N; CPM, x=15.3 N) measures. The latter results indicate that the greatest spasticity occurs in subjects with impaired movement.

522.6 MODULATION OF H-REFLEX FOLLOWING SURFACE SKIN ELECTRICAL STIMULATION, XYLCOCAINE ANESTHESIA AND PLACEBO ANESTHESIA IN CONTROL AND SP EXPERIMENTAL SUBJECTS. A. V. Belanger, A. B. Arsenault, M. L. Durand*, and L. Fortin*. Research Center, Montreal Rehabilitation Institute, Montreal, Quebec, Canada.

The purpose of this study was to determine, in 12 subjects, the extent of soleus motoneuron excitability during conditions of stimulation (Stimulation parameter: S), decreased (Xylocaine Anesthesia, XA) and normal (Placebo Anesthesia, PA) cutaneous inputs. Skin ES was applied using a TENS unit, with the two pairs of electrodes placed respectively over the Achillies (SI dermatome) and TA (IS) tendons. True and placebo anesthesia were respectively obtained after rubbing some Xylocaine (5%) and Vaseline ointment on the skin surface overlying the Achilles tendon. Sets of 10 H-reflexes (Hmax/2) were evoked (1 shock/30s) and averaged at different time intervals before, during and after the testing condition (Hmax values were stabilized during testing). The results, apart from showing a small (10%) H-reflex facilitation during Achilles tendon ES, revealed PA to cause the same gradual facilitatory response (up to 100% after 50 minutes) as that obtained during XA. This finding would appear to seriously challenge the view that reducing (via true anesthesia) neural activity from the skin to the soleus motoneurons significantly increases their excitability. We postulate that the elicitation of consecutive H-reflexes per-se (1 every 30s for minutes) facilitated the H-reflex over time.

Funding: Montreal and Laval University, and Medtronic Inc.
523.1
DESENSITIZATION OF THE ALPHA-2 ADRENERGIC RECEPTOR IN HT29 CELLS. S.B. Jones* and D.B. Bylund (SPON: J.L. Lewis), Department of Pharmacology, Univ. of Missouri, Columbia, MO 65212.

The alpha-2 adrenergic receptor is coupled in an inhibitory manner to adenylyl cyclase. Preincubation with an alpha-2 adrenergic agonist would be expected to result in a shift in the dose response for inhibition upon a subsequent exposure of cells to that agonist. HT29 human colon adenocarcinoma cells have alpha-2 adrenergic receptors which are negatively coupled to adenylyl cyclase. Utilizing the [%]Hadenosine prelabeling technique, we preincubated cells for 30 min with norepinephrine and then stimulated with forskolin and various concentrations of UK14,304, an alpha-2 agonist. Preincubation with norepinephrine resulted in a rightward shift in the dose response curve to UK14,304 resulting in a 20-fold increase in the EC_{50} for UK14,304 (3 to 60 nM). Because preincubation of HT29 cells with norepinephrine results in a 10-fold increase in forskolin-stimulated cyclic AMP production, we repeated the experiment utilizing VIP in the stimulation phase of the assay instead of forskolin. Norepinephrine preincubation again caused a dose-dependent rightward shift in the dose response curve to UK14,304 indicating desensitisation of the alpha-2 adrenergic receptor in HT29 cells. (Supported by NIH GM17664.)

523.2
ALPHA-ADRENERGIC RECEPTORS DOWN-REGULATION BY ALPRAZOLAM. Tyrone Lee, B. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1B8.

Recent reports have shown that alprazolam, a triazolobenzodiazepine, is effective in treating depressed patients with potency equivalent to that of other tricyclic antidepressants. In our earlier attempt to screen for drug action on various receptors in the CNS (Lee et al., Soc. Neurosci. Abst. 12:1080, 1986), alprazolam was found to reduce alpha-2 adrenergic receptor density. The present report investigates the dose-effect relationship of this action.

Six groups of male Wistar rats of 12 each were injected with daily doses of either saline (1 ml/kg) or desipramine (5 mg/kg) or alprazolam (1, 2.5, 5, 10 mg/kg) for 21 days. Brain alpha-2 adrenergic receptor density was determined by Scatchard analysis using [*H]phenylephrine and phentolamine. The results are as follows:

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control</td>
<td>159 ± 6</td>
</tr>
<tr>
<td>Desipramine (5 mg)</td>
<td>142 ± 4</td>
</tr>
<tr>
<td>Alprazolam (1 mg)</td>
<td>141 ± 4</td>
</tr>
<tr>
<td>Alprazolam (2.5 mg)</td>
<td>131 ± 4</td>
</tr>
<tr>
<td>Alprazolam (5 mg)</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>Alprazolam (10 mg)</td>
<td>140 ± 4</td>
</tr>
</tbody>
</table>

It is concluded that alprazolam significantly reduced the density of the alpha-2 adrenergic receptors at all doses attempted in this study. (Supported by the Clarke Institute Research Fund.)

523.3

We have observed that increases in [cAMP] induce an increase in the number of alpha-1 adrenergic receptors (a1-R) in the DDT-1 smooth muscle cell line. a1-R number, measured by the binding of [3H]prazosin, reached 30 to 80% above control after 20 hours of drug treatment. The appearance of new receptors correlated with an increase in a1-R stimulated turnover of inositol phosphates (PI).

Adenosine at micromolar concentrations of a1-R receptors. We have observed that A1 agonists downregulate a1-R in DDT-1 cells by 20 to 30%. Conversely, A1 antagonists upregulate a1-R by 20%, suggesting the existence of two adenosine receptors tonically downregulating a1-R.

Since cAMP upregulates a1-R and since a1 agonists are negatively coupled to cAMP production adenosine could have been downregulating a1-R by decreasing the basal [cAMP]. However, downregulation of a1-R by adenosine agonists still occurred in the presence of 8-bromocAMP. As an alternative mechanism, acute administration of A1 agonists potentiated PI turnover in these cells. Thus, although A1 agonists do inhibit cAMP production and cAMP downregulates a1-R, adenosine may heterologously downregulate a1-R by enhancing the activity of a feedback mechanism involving PI and diacylglycerol. Supported by GM31155.

523.4

Estrogen treatment modified stimulation of [*H]inositol phospholipid (PI) hydrolysis in slices prepared from rat hippocampus, corpus striatum and cerebral cortex. This effect was sex-dependent and required repeated injections of the steroid. Since the estrogen injection protocol (100 ug/animal subcutaneously, once every two days) [*H]inositol monophosphosphate ( [*H]InsP) production in response to NE in hippocampal slices, while an increased responsiveness was found in the cerebral cortex. No effect was observed after a single injection of estradiol benzoate or after an intraperitoneal injection of 17-beta-estradiol to brain slices. In chronic treatment, the steroid response to NE was increased in the hippocampus with no change in the cerebral cortex. The most dramatic changes induced by repeated estrogen treatment were observed in the corpus striatum, where the stimulation of [*H]InsP production by NE was drastically reduced in both sexes. We are currently investigating whether this effect of estrogen at striatal level may be correlated to the antidykinetic properties of the steroid.

523.5
MELATONIN’S INTERACTION WITH beta-RECEPTORS IN RAT PINEAL. T. Karchowski and L. M. Wiles (SPON: G.M. Brown). Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3T5

Binding studies in our lab have suggested an interaction between norepinephrine and [*H]Melatonin binding sites in rat hippocampus (1). In order to clarify this issue, the effects of melatonin on the binding of [*H]beta-adrenergic antagonists ([*H]iodocyanopindolol ([*H]ICYP) and [*H]Ibodipyrrolidone ([*H]IDP) were examined.

Saturable kinetic studies indicate that melatonin does not alter [*H]IDP binding in rat brain. When incubated with homogenates from rat pineal, melatonin enhanced [*H]ICYP binding up to 50% above control values. This effect was dose-dependent and saturable. In agreement with previous studies in rabbit pineal (2). Preliminary findings suggest that 100 nM melatonin significantly enhances the affinity of [*H]ICYP sites in rat pineal.

Control: Kd = 0.20 nM, Bmax = 1090 fmol/mg protein; Melatonin: Kd = 11 nM; Bmax = 930 fmol/mg protein.

Additional studies are required to confirm these findings and to determine the possible mechanisms involved in mediating melatonin’s effects.


523.6

Previous studies suggest that a bromoacetamido congener of 8-hydroxycarbostyril (C-Br) is a potent beta-agonist which may bind to the beta-adrenoceptor in an irreversible manner. In the present study, the effects of in vivo administration of C-Br on the beta-adrenoceptor of several tissues was determined. Similar to in vitro experiments, C-Br (0.1 and 5 mg/kg) induced a shift in the dose response curve in rat hippocampus, corpus striatum and cerebral cortex. In chronic treatment, the steroid response to NE in hippocampal slices, while an increased responsiveness was found in the cerebral cortex. No effect was observed after a single injection of estradiol benzoate or after an intraperitoneal injection of 17-beta-estradiol to brain slices. In chronic treatment, the steroid response to NE was increased in the hippocampus with no change in the cerebral cortex. The most dramatic changes induced by repeated estrogen treatment were observed in the corpus striatum, where the stimulation of [*H]InsP production by NE was drastically reduced in both sexes. We are currently investigating whether this effect of estrogens at striatal level may be correlated to the antidykinetic properties of the steroid.
DESENSITIZATION OF THE BETA-ADRENERGIC SYSTEM WITH AN IRREVOCABLE DUAL-PIGMENTED AGONIST: AN IN VITRO STUDY USING DDT-MF2 CELLS. C.A. Stockmeier and K.J. Kellar. Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

We recently reported on the characterization of a bromocryptamid coengent of (6-hydroxydopamine (C-BR). Our results showed the compound to be a beta-agonist that may bind irreversibly and produced sustained activation effects in vitro (Proc. Natl. Acad. Sci. USA 13:148, 1987). In the present study, the effects of C-BR were investigated using the intact cultured cell system DDT-MF2. Competitive binding studies using [125I]iodocyanopindolol (ICYP) in the presence of 100 µM Gpp(NH)p showed that C-BR was 16 times more potent than (-)isoproterenol (iso). Measurements of cAMP accumulation in intact cells revealed C-BR is 9 times more potent than iso, but equally efficacious. A time course of C-BR accumulation revealed that both iso and C-BR induced a CAMP peak level 3 min following a similar decline, which nonever reached basal levels by 60 min. Addition of propranolol at 3 min resulted in a rapid drop of CAMP to basal levels in the iso-induced system, but had no effect on the C-BR induced one. A time course of receptor loss with 10 µM iso or 1 µM C-BR treatment of intact cells was measured with [3H]CGP-12177. Both iso and C-BR induce a time-dependent loss of receptors as measured on the cell surface. Cells assayed 24 hr later revealed that binding sites from iso treated cells had nearly returned to control levels, whereas the C-BR treated cells still showed an 80% loss of sites. These results imply that C-BR, like iso, causes a desensitization in intact cells. Treatment with cells with iso for 60 min causes a redistribution of receptors, while treatment with C-BR causes loss of binding sites that has not reversed by 24 hr.

DMI TREATMENT DOES NOT DECREASE NE STIMULATED ADENYLATE CYCLASE ACTIVITY IN RAT CORTICAL SLICES FROM LONG-TERM NEURONAL DEPLETED ANIMALS. M.V. Dudley and B. Baron. Merrill Dow Research Institute, Cincinnati, OH 45215

Beta-receptors in cortical membranes and cAMP production in cortical slices were measured 35 days after treatment with xylazine (XYL). XYL (20 mg/kg) produced a sustained decrease in cortical NE, DRG and MPG. Presynaptic markers indicated no loss of neuronal integrity or number. Dose response curves with the non-selective agonist NE on cAMP formation showed the loss of NE antagonized the normal desipramine (DMI) induced (10 mg/kg/days 23-35) increase in maximum response. The inclusion of prazosin (1 µM) or phentolamine (5 µM) in the NE dose-response study in these tissues resulted in the expected decrease in maximal cAMP levels. Neither prazosin nor phentolamine had an effect on the NE dose response in control or the NE-depleted animals. The loss of NE did not affect the number of the selective agonist affinity for ø- or ø-receptors. The loss of NE did not effect basal ø-receptor number or the dose response stimulation of cAMP formation by the selective ø-agonist isoproterenol (ISO). DMI significantly decreased ø-receptor number and the maximum response to ISO on cAMP formation in control and NE-depleted animals. Recently published data has shown that central ø-receptors can modulate ø-receptor desensitization and activation. A chronic NE deficit may effect the coupling of ø-receptors to their second messenger systems which may be important in the postsynaptic effect of antidepressant treatment.

ELECTROSHOCK SHOCK (IES) DECREASES BETA-ADRENERGIC RECEPTOR BINDING DESPITE SCARCE LINEAGING. C.A. Stockmeier and R.L. Kilic. Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106; Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

Dependent desensitization of IES causes a decrease in the number of antagonist-labelled beta-adrenergic receptors in rat cortex (Magarinos and Kellar, 1979). It has been proposed that serotonin causes a play a critical role in this process (Haseloff et al., 1984). We have examined the influence of 10 days of repeated daily IES on [125I]iodocyanopindolol binding sites for which imipramine has high and low affinity in control rats and in cortices from rats treated with 6,7-dihydroxytryptamine (6-DHT) or saline (sham-lesioned). The effects of IES were examined in 6-DHT lesioned rats and control rats. The results indicate that chronic IES treatment decreases receptor binding in both control and 6-DHT lesioned rats. In the control rats, there is a 40% decrease in beta-1 receptors in 6-DHT lesioned rats and in the control rats. In the 6-DHT lesioned rats, there is a 50% decrease in beta-1 receptors.

ONTICHOLOGY AND CHRONIC ANTIDEPRESSANT TREATMENT OF 6-CHD AND 2-ADRENERGIC RECEPTOR MESSQUE RIN IN RAT BRAIN. J.E. Duman and J.F. Talman. Dept. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508

Previous studies have characterized the ontogenic and chronic antidepressant regulation of beta-adrenergic receptors (BAR) by binding analysis. However, little is known about BAR regulation at the level of gene transcription and translation. In the present study we examine mRNA levels for both the beta1 and beta2 subtypes in rat brain. Control and 2 weeks beta-agonist treatment with 2 or 3 rats were divided into two groups. One group received [3H]CGP-12177. The other group received [3H]CGP-12177 and the autoradiograms were scanned and quantitated by a microdensitometer. The results indicate that chronic antidepressant treatment decreases receptor binding in both control and 6-DHT lesioned rats. In the control rats, there is a 40% decrease in beta-1 receptors in 6-DHT lesioned rats and in the control rats. In the 6-DHT lesioned rats, there is a 50% decrease in beta-1 receptors.

PROLONGED ELECTROSHOCK CHANGES THE COUPLING BETWEEN ø- AND ø-ADRENERGIC RECEPTORS IN RAT BRAIN. A. Pilc, J. Balsem and J. Vetulani. Department of Pharmacology, Polish Academy of Sciences, Krakow, Poland and 1 Nova Pharmaceutical Corp., Baltimore, MD 21224.

The cyclic AMP response to ø-adrenergic agonists is greatly enhanced by ø-adrenergic stimulants suggesting a functional interrelationship between these receptor systems in brain. Chronic administration of antidepressants diminishes this receptor interaction, indicating an association with the response to this drug class (Pilc A. and Enna, S.J., Life Sciences 37:1183, 1985). The present study was undertaken to examine whether electroshock, the most efficacious antidepressant therapy, modifies the coupling as well. Male Sprague-Dawley rats were exposed to electroshock once daily for 7 consecutive days after which cyclic AMP and inositol phosphate (IP) accumulation were examined in cerebral cortical slices using prelabeling techniques. Whereas electroshock treatment had no effect on imipramine-stimulated IP accumulation or on norepinephrine-stimulated IP accumulation, it significantly reduced the cyclic AMP response to norepinephrine alone and to imipramine in the presence of 6-fluoromephenamine, an ø-adrenoceptor agonist. The results suggest that antidepressants modify the functional coupling of ø-adrenoceptors in an ø-adrenergic site distinct from that associated with inositol phosphate production.
523.13

HUMAN LYMPHOCYTE BETA ADRENERGIC RECEPTOR DENSITY - POSSIBLE RELATIONSHIP TO PANIC-AGORAPHOBIC SYMPTOMS IN DEPRESSED WOMEN.

J. Maglizio, B.W. Gietse, B. Maddock* and A. Doren*.
Dept. Psychiatry Sch. Med. Univ. Calif., Davis, CA 95616

To investigate the relationship between lymphocyte beta-adrenergic receptor density (BMX), binding affinity (KD) and psychological symptoms in depressed outpatients, both were utilized in a multivariate regression model to predict pretreatment scores of the Hamilton Depression Rating Scale (HAM 17), Beck Depression Inventory (BDI), Sheehan Patient Rated Anxiety Scale (SPRAS), State- Trait Anxiety Inventories (XI and X2) and Chambliss' Accompanied (MOBACC) and Unaccompanied (MOBUL) Mobility Inventories. Forty-six outpatients with major depression, unipolar type (DSM-III-R), (20 females, 26 males) participated. Binding was performed on partially purified lymphocyte membranes using the antagonist ligand 125[I]-iodocyanopindolol (ICYP).

In female patients, BMX negatively associated with scores on both Mobility Inventories (MOBUL; b'=-0.722; p=0.043; MOBACC; b'=-0.913; p=0.002, Lambda=0.181; p=0.005), but was of no predictive value towards the other measures. In males, neither BMX nor KD predicted scale scores. These results provide preliminary evidence of an association between lowered lymphocyte beta-receptor BMX and severity of panic-agoraphobic symptoms in female depressed outpatients.

524.1

INTRACEREBRAL ADMINISTRATION OF ADENOSINE TO THE MEDIAL PREOPTIC AREA ENHANCES SLEEP IN RATS.

Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

To assess the role of adenosine in the medial preoptic area in the sleep-wake cycle of the rat, we have intracerebrally administered adenosine at doses of 5, 25 and 50 nmol. All doses significantly increased total sleep by 27%, 38% and 28%, respectively, during the 6 hr polygraphic recording period. Analysis of the various sleep states revealed that the enhancement in total sleep was due to a proportional increase in SW_1 (p<0.01), SW_2 (p<0.01) and REM (p<0.01) sleep. In addition, doses of 5 and 25 nmol significantly decreased sleep latencies for all three sleep stages (p<0.01). These observations support the hypothesis that adenosine in the medial preoptic area may be involved in the regulation of sleep in rats. Supported by FED-AFOSR contract 85-0349 to MR.

524.2

CHARACTERISTICS OF ADENOSINE A_1 RECEPTORS AND ADENOSINE A_3 UPTAKE SITES IN THE BRAIN OF NARCOLEPTIC DOGS.


In order to assess the role of the adenosinergic system in narcolepsy we have examined (3H)-R-PIA binding to adenosine (ADO) A_1 receptors and (3H)IBI binding to ADO uptake sites in cerebral cortical membranes isolated from normal and narcoleptic canine brains. Scatchard analysis of (3H)-R-PIA binding in sigmoid and coronal gyrus membranes isolated from normal and narcoleptic dogs revealed a single class of high affinity A_1 receptor sites. Our preliminary data also suggest a trend for A_1 receptor upregulation in both cortical gyri of narcoleptic dogs. This is of interest since ADO A_1 receptor upregulation has been found following REM sleep deprivation in both cortical gyri of narcoleptic dogs. This is of interest since ADO A_1 receptor upregulation has been found following REM sleep deprivation in both cortical gyri of narcoleptic dogs.

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524.3

LOCALIZATION OF A SLEEP INDUCTION SITE WITH SHORT LATENCY BY MICROINJECTION OF CARBAZOL IN A HEAD-RESTRAINED CAT.

Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.

In an attempt to identify a neuroanatomical region which can be chemically stimulated to induce D sleep with very short latency, four cats were placed in a chronic stereotactic apparatus and microinjected with carbazol (4ug/250nl) using a movable microinjector assembly (Yamamoto et al. 1988). The time from injection until the first D episode was scored and correlated with injection site. Our results indicate that shortest latencies were found when carbazol was injected in the rostral extent of the anteromedial pons, a region contiguous with nucleus cuneiformis, central grey, and mesencephalic tegmentum. A map of latency vs. injection coordinate indicates this D induction site is highly localized, since injections greater than 0.5 mm away from this site yield significantly longer (>8 min.) latencies. These results suggest that this region represents a near optimal intersection of multiple neural networks involved in D-sleep generation, such that many networks are simultaneously activated by the diffusion of carbazol. Supported by NS 13923.

524.4

EFFECTS OF LY33837, A SELECTIVE 5HT-2 ANTAGONIST ON SLEEP IN THE RAT. R.H. Pattei, Physiology & Behavior Branch, Dept Medical Neuroscience, WRAIR, Washington, DC 20307.

Recently, we have studied the effects of 3 5HT-2 antagonists (Ritanerin, IC1 163,369 & IC1 170,809) on sleep in the rat (1,2). All three suppressed REM and increased REM latency. Neither IC1 compound had an effect on NREM, but Ritanerin disrupted NREM. Others have reported an increase in NREM following ritanserin (3). The present study examines the effects of LY33837, another selective 5HT-2 antagonist. Four male S.D. rats were given saline (ip) and 24 hr later LY33837 (1 mg/kg, ip). Injections were at light onset and sleep was measured for 6 hr after injection. NREM latency was increased in hr 2-4 after injection and REM was decreased from hr 2-5 following injection. NREM latency was not changed, but REM latency was increased. A decreased number of REM periods was also noted. The present study supports previous findings of REM suppression and increased REM latency following administration of 5HT-2 antagonists. However, the effects of 5HT-2 antagonists on NREM sleep are not consistent. We and others (3) have found an increase in NREM sleep, but in previous studies (1,2) we have found no change in or a disruption of NREM. The role of 5HT-2 receptors in sleep mechanisms is still not resolved.
524.5 INFECTION CHALLENGE ALTERS SLEEP IN RABBITS. L.A. Toth and J.M. Kvetnansky. Comparative Anatomy and Physiology, Univ. Tennessee, Memphis, TN 38163. We recently described altered sleep in rabbits after inoculation with Staph. aureus, a gram-positive bacteria (Neurosci. Abstr. 13: 261, 1987). We now report that sleep is also altered in rabbits inoculated with E. coli, a gram-negative bacteria, and Candida albicans, a fungal organism. E. coli increased both slow-wave sleep (SWS) and EEG slow-wave amplitudes (SWA) for the first 4 h postinoculation (PI). SWA was then decreased for the next 20 h. Similar effects occurred if heat-killed E. coli were injected. In contrast, Candida enhanced both SWS and SWA from 6 to 18 h PI and decreased them from 24-48 h PI. Sleep was not altered after inoculation with heat-killed Candida. Both E. coli and Candida inhibited REM sleep. Sleep responses induced by Candida and Staph. inoculations are similar and may be related to immune stimulation of the host. The more rapid effects produced by E. coli may be related to the presence of heat-stable endotoxin in the cell wall of this organism. (Supported by NNR-9001A-83-K-0773 and NIMH-225378)

524.6 RATES OF CEREBRAL GLUCOSE UTILIZATION IN THE RAT ARE HIGHER DURING REM THAN DURING SWS. R.C. McGuity, R. Drucker-Colin, Spence, J. Velázquez-Nocentia, Dept. de Neurociencias, Instituto de Fisiologia Cellular, UNAM, Mexico. It has been reported that auditory and somatic stimulation during rapid eye movement (REM) sleep is capable of increasing REM sleep duration. The present work attempts to determine whether changes in REM unit activity are related to this phenomenon. Two cats were implanted under pentobarbital anesthesia with electrodes for conventional polygraphic recording. Two barrel microelectrodes were targeted into the MRF. The sleep-wake cycle was recorded alternating control REM sleep periods with auditory stimulation (80 dB, every 20 sec) and the units discharge rate was determined. 17 Cells were recorded while 7 units (41%) were not responsive to stimulation; 10(58%) were responsive to the stimulus. 7 of these did not show changes in their discharge rate when comparing control vs stimulated periods. Three cells increased the mean discharge rate during stimulated periods (p < .05).

524.7 VIP-INDUCED REM SLEEP ENHANCES LEARNING IN PCPA PRE-TREATED RATS. R. Alam, P.M. Baxter, C. Hallgren, G. Marini*. I. Gritti and M. Mancia (SPON: European Brain and Behavior Society) 1st. Fisiologia Umana II & ITBA via Mangiagalli 32-M ilano-Italy. It has been shown that VIP-induced REM sleep enhances learning in rats. Passive avoidance tests showed that VIP group had a prolonged step-through latency with respect to saline group (342.5 ± 96.5 sec vs 28.5 ± 16.5 sec). Various results strongly suggest that VIP-induced REM sleep enhances learning in rats.

524.8 MEDIAL RETICULAR FORMATION (MRF) UNIT ACTIVITY DURING AUDITORY STIMULATED REM SLEEP PERIODS. J. Lantos, D. Ralston, C. R. Holmes, M.H. Webster*, R. Stinson and B.R. Jones, Department of Neurology & Neurosurgery, Montreuil Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 1N4. Since the classical neurophysiological studies of Magoun and Milner, it has been assumed that the motor inhibition occurring during paradoxical sleep depends upon neurons in the ventromedial reticular formation (VMRF) which project to the spinal cord. To test this assumption, we have selectively recorded microelectrodes in this area with stereotaxic injections of quisqualic acid (90ug) in cats implanted with standard microelectrodes for E.E.G., P.S.G. and EMG. The animals were recorded 24 hours per day one week prior to and one month subsequent to the lesion. The daily time spent in waking, slow wave sleep and REM sleep was not greatly altered, but neck muscle tonus was significantly increased in PS during the full month of recording following the lesioning surgery, and this increase was associated with whole body movements, including vigorous movements of the limbs. Histological analysis of the tissue revealed extensive cell loss in the VMRF. These results support the contention that the cells of the ventromedial reticular formation contribute to muscle atonia during paradoxical sleep in the cat. (Supported by MMC of Canada)
BIOLOGICAL RHYTHMS: SLEEP

524.11


In experiments utilizing bilateral, chemical or electrical stimulation of the medullary rostral reticular formation, medullary neurons have been identified in which stimulation produces a marked atonic response in the hindlimb. We have previously found that within the medullary pyramid, there is a subpopulation of neurons which exhibit a marked atonic response to electrical stimulation and which is associated with negative intracellular potentials. We have investigated the cellular basis of atonia in the medulla and have identified a subpopulation of medullary neurons which exhibit a marked atonic response to electrical stimulation.

524.12


We have examined the effects of tone intensity on PGO waves during REM sleep. PGO waves were elicited by a series of tones at different intensities (60, 70, 80, 90, and 100 dB) and each block of 8 sessions was tested for on-line computer analysis. We have found that PGO waves are more common at low intensities and are more frequent at higher intensities.

524.13

SUPPRESSION OF MUSCLE TONE PRODUCED BY ELECTRICAL STIMULATION OF THE MEDULLARY Reticular Formation. Y. Y. Siegel and Y. Lai. Sepulveda VAMC, UCLA School of Medicine, Sepulveda CA 91343

We have previously reported that suppression of muscle tone can be produced from three brainstem sites impalced in REM sleep. Acetylcholine (ACh) microinjected into the dorsolateral pons (DLP) produces a suppression of muscle tone at the ponto-mesencephalic junction. In the present study, we have investigated the mechanism of this suppression.

524.14

RECEPTORS MEDIATING SUPPRESSION OF MUSCLE TONE PRODUCED BY GLUTAMATE IN DORSOLATERAL PONS AND MEDULLA. B. N. Mallick and J. M. Siegel. Sepulveda VA MC, UCLA School of Medicine, Sepulveda CA 91343

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524.15


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524.16


The occurrence of postictal-potentiated, the neuronal substrates of these changes are unknown. In the present study, we have taken advantage of the extraordinary stability of the micro-circuit recording technique to analyze changes in unit activity during REM deprivation. We have studied 8 cells in the dorso-lateral pontine reticular formation. The results indicate that the neuronal activity in the pontine reticular formation may be involved in the generation of REM sleep.

524.17


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BIOLOGICAL RHYTHMS: SLEEP

by period amplitude analysis, Fourier analysis, etc. Data from these analytical techniques are usually subjected to misleading statistical evaluations which are limited by their inability to statistically control multidimensional data sets. Therefore, we combined the powerful features of single case design, derived power spectral parameters, factor analysis and computerized three dimensional graphics in the development of EEG factor scans. Frontal-pairtial cortical EEG recordings were collected during sleep-awake cycles, drug-induced states and states of narcolepsy in rats. Quantitatively and qualitatively unique EEG factor scan patterns were generated from each of these CNS states. As an example, the relative theta, kapa and sigma power of EEG activity in rats and hypothalamic sleep patterns were distinguished by the patterned loadings of several factors which are independent of age two sleep-wake state differences. The EEG factor scan patterns are expected to have utility in the classification of disparate groups of CNS active agents and pathological states. (Supported in part by USPHS grants NS02670 and NIDA DA01050)

RESPIRATORY DISTURBANCE INDEX CHANGES WITH AN ANTERIOR MANDIBULAR POSITIONING DEVICE FOR OBSTRUCTIVE SLEEP APNEA. B.T. Clark, D. Arand* and E. Chung* (SPHN; M.C. Carter). UCLA Dental Research Inst. & Sleep Disorders Clinic, Neuropsychiatric Inst., Los Angeles, CA 90024.

Several case reports have indicated that dental devices may have a potential effect in the reduction of obstructive sleep apnea (OSA) problems. This study utilized an anterior mandibular positioning appliance in OSA patients and evaluated the changes in their respiratory disturbance index (RDI). The RDI is calculated by dividing the total sleep time into the total number of oxygen desaturations. The amount of movement induced by the device was 60-80% of the mandible's maximum protrusive distance (5-7mm) measured from maximum incisal closure. The RDI levels were determined during all night polysomnographic studies. To date, 5 diagnosed OSA subjects have completed all phases of the protocol which involved a pre-appliance sleep study, a 1-month post-appliance sleep study, and a 6-month post-appliance clinical examination. All 5 subjects showed a clear decrease and the mean RDI level for the RDI pre-appliance was 28.3 ± 7.3 and 4.2 ± 1.93 post-appliance. The 6-month clinical examination data clearly indicates that no adverse change in jaw function (range of motion, joint sounds or muscle tenderness) and patients continue to be fully compliant with the prescribed all night use of the appliance.


Sleep onset on the multiple sleep latency test (MSLT) was determined using a single epoch of stage 1, a single epoch of stage 2 or REM sleep for 21 patients (mean age 38.0 yr) with narcolepsy and 21 patients (mean age 38.0 yr) with obstructive sleep apnea (mean apnea index of 64.3). The mean MSLT procedures were from 10:00 to 18:00. The resulting polysomnograms were scored in 30 sec epochs. For patients with narcolepsy, mean sleep latency in min using one epoch of stage 1 was 3.07±0.35, using three epochs of stage 1 was 3.37±0.39, and one epoch of stage 2 or REM was 5.07±0.55 (p<.001). The respective values for patients with sleep apnea were 3.61±0.54, 4.36±0.61, and 8.62±0.85 (p<.001). Measures of sleep latency were intercorrelated for patients with narcolepsy (r=.701, p<.001) and for patients with sleep apnea (r=.894 to .951, p<.001). The obstructive apnea prevented or delayed sleep onset 18.14% of trials using one epoch of stage 1, 24.76% using three epochs of stage 1, and 27.62% using one epoch of stage 2 or REM criteria for patients with sleep apnea. A single epoch of any stage of sleep is an appropriate measure of sleep latency for the pathology-related interruptions.

EGG: CHAOS AND QUASIPERIODICITY. H. Stowell. ERFB Lab., 120 Nature Creek, Milledgeville GA 31061.

DEGENERATIVE DISEASE: PARKINSON'S (NONPRIMATES)

525.1 AGGREGATE CULTURES OF RAT FETAL DOPAMINE (DA) NEURONS REVEAL EXPANDING BEHAVIORAL EFFECTS OF PARKINSONIAN NUDANCE IN RATS. P. Loring R.E. Streeker, B. Boss, R. Mail.* Hana Biologics, 850 Marina Village Parkway, Alameda, CA 94501

Neural grafts of cultured dissociated DA-rich fetal ventral mesencephalon (VM) restore function in rats made parkinsonian by 6-OHDA treatment. If graft therapy for Parkinson's disease is to be successful, it may become important to preserve fetal tissue integrity during culture. However, it is likely that neurons that grow processes onto planar substrate suffer damage during dissociation required to remove them from culture dishes for grafting. To avoid this problem, we have used aggregate tissue culture methods, which allow cultured DA-rich neuronal tissue to be grafted after only one initial dissociation procedure. Donor tissue was dissected from the VM of 13 day old rat fetuses and prepared for aggregate culture by a modification of the method of Hemmendinger et al. (PNAS 78:1204-1208; 1981). Cells were cultured 9 days in rotating flasks. The aggregates formed many small balls (175-300 um dia.) each estimated to contain over 6,000 cells. Forty such balls were injected via a 22 G needle into the DA-denervated striata of host parkinsonian rats. Six weeks post grafting 7 of 11 rats exhibited behavioral signs of a functional graft, as shown by both reduction in amphetamine-induced ipsilateral rotation and increase in contralateral rotations. One rat sacrificed at 3 weeks post grafting had over 400 surviving dopaminergic neurons, judged by tyrosine hydroxylase (TH) immunohistochemistry. TH-positive fibers extended from the transplant site into the host striatum. These results suggest that aggregate culture methods are promising means to maintain and deliver fetal neurons for graft therapy.

525.2 ENCAPSULATED EMBRYONIC MOUSE MESENCEPHALIC TRANSPLANTS ALLEVIATE EXPERIMENTAL PARKINSONISM IN RATS. P. Aebersch* S.R. Winn* D. Ross (SPON: J. Parmelee). Artificial Organ Laboratory, Brown University, Providence, RI 02912

Embryonic mesencephalon xenotransplants alleviate experimental parkinsonism, however they are rejected over time. We are studying the ability of cognates of mesencephalon and striatum immunoprotected by a permeable membrane to affect movement in parkinsonian rats. An aggregate polymeric membrane with a molecular weight cut-off of 50,000 daltons provides immunoprotection by allowing nutrients, growth factors and neurotransmitters to freely diffuse through the membrane while preventing the invasion of immunoglobulins and cells. Polyvinyl chloride acrylic copolymer permeable tubes, 1D 600 um, were loaded with E14 mouse cognates and implanted with a companion polymetric capsule. The polymer capsules were then placed stereotaxically in the striatum of 6-hydroxydopamine lesioned rats. Rats implanted with empty polymer capsules served as controls. Motor asymmetry was assessed under amphetamine (5 mg/kg) stress. Cohorts of 6 animals were observed for at least 8 weeks. A significant decrease in rotational behavior was observed in 5 of the 6 encapsulated cognates whereas no decrease was observed with empty polymer capsules. Upon retrieval intact neuron-like cells were observed within the polymer capsules up to 3 months of implantation. Tissue reaction to the polymer capsule was minimal. These results indicate that synaptic contact is not required in order to reduce the symptomatology of experimental parkinsonism. Whether the effects observed are due to dopamine release induced by growth factors released from the transplants is not known.

525.3 INTRASTRIATAL IMPLANTATION OF AN ENCAPSULATED L-DOPA RELAISING POLYMER REVIVES EXPERIMENTAL PARKINSONISM IN RATS S.R. Winn, A.N. Salkeld., P. Aebersch* (SPON: N. Knuckle). Artificial Organ Laboratory, Brown University, Providence, RI 02912

Parkinson's disease is a deficiency of dopamine within the striatum. We are investigating the feasibility of implanting a polymetric substrate which releases L-Dopa to reverse experimental parkinsonism. Films of polyethylene vinyl acetate (PEVA) containing 10% by weight of L-Dopa were cast, shaped into cylinders, and encapsulated with perme-selective acryl polymer tubes with a nominal molecular weight cut-off of 50,000 daltons. The tubes were sealed with a compatible polymer glue and implanted stereotaxically into the striatum of 6-hydroxydopamine lesioned rats. Cohorts of 3 animals received L-Dopa loaded capsules; controls received capsules loaded with substrate alone. Rotation behavior was observed under amphetamine challenge (3 mg/kg). Immediately following transplantation, animals with L-Dopa loaded capsules returned to pre-transplant rotational values. One week post-transplantation, rotational behavior in animals with the L-Dopa containing capsules returned to pre-transplant values. No changes in post-transplantational rotational asymmetry were observed in control animals. The present study shows that intrastriatal release of L-Dopa suspended in a hydrophilic polymer is able to reverse experimental parkinsonism in rats. Controlled release of dopaminergic or macromolecules from a polymetric substrate may provide an alternative method of treatment for neurologcal disorders.


Fetal pig CNS is an attractive model system to use for the development of a graft therapy for Parkinsonism in rats. The development time course of the pig more closely approximates that of the human embryo, and pig DA neurons can be expected to innervate a larger brain area than rat neurons. Long-term survival of pig grafts in rats has been shown to require immunosuppression with CyA, a time-consuming and expensive procedure. Hence, we tested whether fetal pig CNS grafts would survive in both nude and immunosuppressed rats. DA rich cell suspensions of freshly dissected embryonic day 21 fetal pig ventral mesencephalon were grafted into the right striatum of 20 CyA-immunosuppressed and 8 nude rats. Control rats were grafted with unilaterally parkinsonian with 6-OHDA. At 9 post-grafting day, 15 of 20 CyA-treated rats exhibited behavioral effects of the grafting, as shown by a reduction of amphetamine-induced rotation of 12-17 weeks. Cessation of CyA treatment resulted in behavioral signs of graft rejection within 4 to 9 weeks. Control grafted rats (N=8) showed no change in rotation. Large numbers of DA neurons (identified by tyrosine hydroxylase immunohistochemistry) were found in functional grafts. Grafted nude rats showed a similar reduction in amphetamine-induced rotation, which remained stable for at least 18 weeks, indicating that the nude rat model is a useful alternative to CyA injections in CNS-xenografting experiments.


For the last several years, we have been systematically studying the spatial and temporal evolution of degeneration induced by MPP\(^+\) in the nigrostriatal model system of Parkinson's disease. This is our first report of fine structural changes. Two days after a single injection of MPP\(^+\) (20 mg/kg sc) into 16 nude rats, 8 of the rats sacrificed 1 and 4 days later. A third group of rats was sacrificed and processed for electron microscopy 15 days after injection of MPP\(^+\) (20 mg/kg sc). The locations of the mesencephalon and striatum of each rat were determined by histology before implantation of the MPP\(^+\).


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In the MPTP treated animals, major changes were seen in the rough endoplasmic reticulum (RER) and mitochondria. Similar mitochondrial changes have been reported in other pathological models in which the neurotoxic effects are thought to be the consequence of calcium overload such as kainic acid poisoning, cerebral hypoxia, and ischemia. We have recently reported that incubation of mouse brain homogenates with MPP\(^+\) and increasing concentrations of calcium produces changes similar to those observed in the MPTP treated animals. The results of these experiments are presented in this paper.
MPTP PRODUCES A MOSAIC PATTERN OF TERMINAL DEGENERATION IN DOG:

DEGENERATIVE DISEASE: PARKINSON'S (NONPRIMATES)

Friday AM

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DEGENERATIVE DISEASE: PARKINSON'S (NONPRIMATES)

Friday AM

H anda, N.S. Canada, B3H 4H7

DEGENERATIVE DISEASE: PARKINSON'S (NONPRIMATES)

Friday AM

H anda, N.S. Canada, B3H 4H7

DEGENERATIVE DISEASE: PARKINSON'S (NONPRIMATES)

Friday AM

H anda, N.S. Canada, B3H 4H7
526.1 CROSS REACTIVITY OF BRAIN REACTIVE AUTOANTIBODIES. C. S. Madan* and D. A. Wolfson. Dept. of Neurology, University of Washington, Seattle, WA.

Autoantibodies to brain membrane antigens have been demonstrated in patients with various neurological diseases. In the process of identifying these autoantibodies, we have encountered several instances of cross reactivity with other brain membrane antigens. This indicates the existence of a common antigenic epitope on various brain membrane proteins.


An antibody was identified in the serum of a patient with Systemic Lupus Erythematosus (SLE) and CNS manifestations which specifically bound to a brain membrane antigen. This antigen (lp50), which appears to be an integral membrane glycoprotein, was not detected in extracts of cardiac tissue. It was highly specific for brain, liver, kidney or pancreas. The serum was screened by Western blotting with lp50 purified from brain synaptic plasma membrane. Serum samples from 13 patients with connective tissue disorders including rheumatoid arthritis, polymyositis, scleroderma, vasculitis, and SLE were tested. Eleven of 21 patients who showed antibody reactivity with lp50 had CNS disease manifested by depression, psychosis or seizures. The 12 remaining patients and 3 normal controls did not express any lp50 antibodies, and had no apparent clinical CNS manifestation of disease.


Brains of ten individuals afflicted with adult onset of dementia showed no neurofibrillary tangles, neuritic plaques, neuritic threads or amyloid deposits but did show argyrophilic grains scattered throughout the neocortex of some cortical areas. The grains were best demonstrated with the Gallyas technique and showed the same structures as in scrapie. The possibility of scrapie agent derived from brain is essential for development of Huntington's-like symptoms by injection of scrapie material into the caudate of mice (Rieke, Scarfi & Hunter, 1984).


Scrape is a neurodegenerative disease of animals and is a model for the human diseases kuru, Creutzfeldt-Jacob disease and Gerstmann-Strassler syndrome. The physical properties of the scrapie agent are not known, however, a 33-37 kDa modified host protein (Sp33-37) derived from infected hamster brain is essential for disease production in animals. The smaller form of this protein (PrP 27-30) is produced from Sp33-37 by partial proteolysis. Chemical modification of specific amino acid residues under mild denaturing conditions was used to probe the protein structure and function. The modified protein was detected using polyclonal and monoclonal antibodies to PrP 27-30 and a monoclonal antibody. 3F4, 3F4 did not bind to the scrapie protein after cleavage at methionine residues or following modification with diethyl pyrocarbonate (DEP). Modification with succinic anhydride, N-succinimidyl 3-(4-hydroxyphenyl)propionate and chloramine-T did not reduce 3F4 binding. Infectedivity was reduced by modification of this amino acid with DEP and restored after reversal with hydroxylamine, implicating histidine as an active site residue. 3F4 binding was not restored by hydroxylamine treatment. Possible sites for the monoclonal epitope are suggested from the sequence predicted from cDNA clones of Sp33-37. NIH Grant NS23948

526.5 IMMUNOPEROXIDASE LOCALIZATION OF CYTOSKELETAL PROTEINS IN SPINAL CORD OF THE MOTOR NEURON DEGENERATION (Mnd) MOUSE. J. E. Callahan* and A. D. Winter. Dept. of Neurology, University of Rochester, Rochester, NY 14642.

We recently reported the presence of phosphorylated neurofilament (pNF) epitopes in the spinal cords of Mnd mice. While only mutants contained somatic pNF, there was no apparent correlation between the number of pNF positive cells and disease pathology. To determine the significance of the pNF staining and to learn more about the pathology of Mnd mice, we used immunohistochemical techniques to label specific cytoskeletal epitopes in the cell soma of spinal motoneurons of the Mnd mouse. We found that pNF correlated with disease pathology. This cross-reactive host protein (Sp33-37) which appears to be a major component of the pNF-reactive epitope, is a novel integral brain membrane protein of a non-autoimmune mouse strain (Balb/C). The membrane proteins included by detergent extraction and phase separation, separated on SDS polyacrylamide gels under non-dissociating conditions and transferred onto nitrocellulose membrane. Sera incubated with the blot and stained with anti-mouse Ig antibody indicate that autoantibodies to brain specific as well as cross reacting antigens can be found. The data and their implications for CNS involvement in autoimmune disease will be discussed.

Supported by the Deutsche Forschungsgemeinschaft.


A recent study has shown that 1-pyrroglutamic acid, a major intermediate in the gamma-glutamyl metabolic cycle, mimics the symptoms of Huntington's Chorea when injected unilaterally into the caudate of mice (Johansson, 1984). In contrast, we have observed that 1-PG is not toxic to Mnd mice. In preliminary studies we are looking at the development of Huntington's-like symptoms by injecting 1-PG into the caudate of mice (Rieke, Scarfi & Hunter, 1984).

We examined the effects of age on the development of Huntington's-like symptoms by performing bilateral injections of 1-PGA into the caudate of 6 month old and 2 year old male Wistar rats. Several physiological measures were taken, including two tests of muscle strength, a measure of aggression and generalized behavioral observations. In addition, the rat's footprints were examined in a straight row; evaluation of the distance between the feet, the width of the row, and signs of ataxia, or gait disturbance.

We found several significant differences between young and old rats in response to bilateral lesions. Younger animals displayed significantly increased aggression, while older animals displayed more ataxia & general gait disturbances. In addition, the younger rats ataxia and older rats demonstrated a significant loss of muscle strength following injection. These results suggest that the neurotoxic effects of 1-PGA become more pronounced with age, which may prove helpful when examining the slow progressive degeneration of neural tissue in Huntington's patients.
DEGENERATIVE DISEASE: OTHER II

FRIDAY AM

Huntington's disease (HD) can be replicated by intrastriatal infusion of pharmacological interventions. Ten monkeys received stereotaxic unilateral lesions to the primate by excitotoxic lesions of the caudate-putamen complex (CPU) in CEA, Orsay 91406, France.

One showed intense TH immunoreactivity but no AChE reactivity. The other was moderately reactive to TH and showed intense AChE staining. Electronmicroscopically these two cell populations were clearly distinguished. AChE reactivity was observed only in the neuron-like cells, in which the reaction was mainly located in the endoplasmic reticulum.


In the rat, many of the neuropathological and neurochemical features of Huntington's disease (HD) can be replicated by intrastriatal infusion of excitotoxic substances such as ibotenic acid. We have developed a lesion model of HD in the primate by excitotoxic lesions of the caudate-putamen complex (CPU) in order to test treatment strategies such as neural transplantation and pharmacological interventions. Ten monkeys received stereotaxic unilateral infusion of ibotenic acid into the right CPU, while four unoperated animals served as controls. Three animals received stereotaxic implantation of fetal cell suspensions prepared from striatal primordia into the lesioned CPU. These monkeys were treated with the immunosuppressant drug cyclosporin. Two to 35 weeks after the lesion the animals were assessed in their home-cages for spontaneous behaviours and motor abnormalities induced by various doses of the dopamine agonist apomorphine (0.5, 1.0 and 2.0 mg/kg). Behavioural studies indicated few spontaneous abnormalities following the unilateral ibotenic acid lesion, but injection of the dopamine agonist drug induced dramatic dyskinesias and motor stereotypes in the lesioned animals, while controls were either not affected or showed mild stereotypic motor behaviours. In vivo PET studies indicated clear lesion-induced changes in the CPU, such as reduction of dopamine receptor binding. Anatomical and histological analysis showed striatal lesions that corresponded closely to the anatomical distribution of CPU damage seen in HD. Results in progress describing the effects and survival of the transplants will be presented at the meeting.

REGULATION OF AUTONOMIC FUNCTION III

LIGHT AND ELECTRON MICROSCOPICAL OBSERVATIONS OF ACRYCHOLINESTERASE REACTION IN DEVELOPING RAT RETROPERITONEAL PARAGANGLIA. Liisa Eräkön, Naiset Anonen and Nila Bäck. Department of Anatomy, University of Helsinki, Finland.

The main retroperitoneal paraganglia of the newborn rat consists of different kinds of catecholamine cells; one with moderate immunofluorescence of dopamine beta-hydroxylase (DBH) resembling sympathetic nerve cells; another with bright fluorescence to TH and DBH. The latter type are smaller and are considered as paraganglion type cells.

The developing paraganglion cells initiated catecholamine synthesis on the 13th prenatal day. At this stage, some of these cells showed AChE reaction. During later developments, two subpopulations of cells were distinguished. One showed intense TH immunoreactivity but no AChE reactivity. The other was moderately reactive to TH and showed intense AChE staining. Electronmicroscopically these two cell populations were clearly distinguished. AChE reactivity was observed only in the neuron-like cells, in which the reaction was mainly located in the endoplasmic reticulum.


Autonomic ganglia in the frog are traditionally described as simple relay stations. However the variety of peptides found in ganglion cells and their preganglionic inputs suggests that these ganglia may be more complex. In an effort to define the microenvironment of identified cells after studying their responses to particular peptides, we began to label the cells by intracellular injection with HRP. We found that the morphology of both sympathetic lumbar chain cells and parasympathetic cardiac ganglion cells in the frog is more complex than the simple unipolar description normally given.

Most HRP-labeled cardiac ganglion cells (located in the interstitial septum) had extensive axon collaterals arising from the axon within 30 μm of the cell bodies. These local collaterals were in addition to the main axonal projection into the cardiac muscle and contained numerous synaptic bouton-like swellings. Local axon collaterals, though less extensive than those on cardiac ganglion cells, were also present on 30% of the cells in lumbar sympathetic ganglia. The cells with local axon collaterals were a subset of the total population of ganglion cells based on size. The largest cells did not have axon collaterals. (PHS RO123978 & NSF BNS 8605611)

IMMUNOREACTIVITY IN CHRONIC DEGENERATIVE NEUROLOGICAL DISEASES: P.B. Stinnett, P.L. McGeer, D.Riche* and M.Maziere*. Dept. of Anatomy, University of Cambridge, Cambridge CB2 3DY, England and Frederic Joliot Hospital, Dept of Biology, CEA, Orsay 91406, France.

Many chronic degenerative neurological diseases are characterized by the appearance of a low grade inflammatory reaction in regions selective for particular disease. Alzheimer’s, Parkinson’s and Huntington’s disease, amyotrophic lateral sclerosis, Shy-Drager syndrome, progressive supranuclear palsy, and Parkinsonism-dementia of Guam are examples. The most prominent immunological finding in these diseases is reactive microglia positive for the M0 class II glycoprotein HLA-DR, which is essential for presentation of foreign antigen to T-lymphocytes. Reactive microglia are also weakly positive for leukocyte common antigen (LCA). LCA positive round cells can be found in vessels and in the matrix of affected areas. Subpopulations of these cells, which can be identified by markers for lymphocytic subsets include T-lymphop suppressor and T-helper/inducer. Reactive astocytes, also present in these conditions, are a separate population from the microglia as shown by double immunostaining for glial fibrillary acidic protein and HLA-DR. The results suggest that cell-mediated immune responses occur in many diseases where such involvement has not previously been suspected. HLA-DR staining may be a valuable addition to standard neuropathological methods.

Supported by grants from the Alzheimer’s Society of B.C., MRC of Canada, and the B.C. Med. Svcs. Fndn.

New autonomic nerve connections from the contralateral superior cervical ganglion (SCG) and middle cervical ganglion (MCG) to SCG were demonstrated by applying the Horseradish Peroxidase (HRP) retrograde tracer technique. HRP was injected into the unilateral SCG in 12 cats. After survival times of 2 or 3 days, the animals were killed and their spinal cord, brain stem and sympathetic ganglia were processed to visualize HRP labeling by using the tetramethylbenzidine method. Labeled neurons were found not only in ipsilateral T1-5 spinal cord segments, SCG and sympathetic ganglia, but also within the contralateral SCG and MCG. Lesions in the sympathetic chain caudal to the SCG injection site prevented spinal cord labeling but did not affect the SCG and MCG labeling. Thus, the contralateral pathway utilizes a rostral course. In conclusion, the presence of the contralateral projection to the sympathetic cervical ganglion challenges the principle of the two-neuron-chain peripheral autonomic nerve system. (Grant NSC 87-0412-B0016-32, Taiwan, R.O.C.)

APPARENT PLASTICITY OF AUTONOMIC NERVE STIMULATION OF THE HYPERPLASTIC PROSTATE: INCREASES IN PENILE PRESSURE AFTER CHRONIC INTERRUPTION OF THE SACRAL PARASYMPATHETIC OUTFLOW. M.P. Olmsted*, G. Walton* and A.E. Brawley* (Supported by NIH grants HL37223 to S.F.E. and 507RR5371 to C.F.M.)

We re-examined the afferent input to pelvic viscera by means of the anterograde transport of horseradish peroxidase (HRP). D. M. Nance. J. Burns*. C. M. Klein*. Investigations have previously documented that preganglionic fibers in the pelvic plexus are under tonic sympathetic inhibition in response to the activation of the pelvic viscera. The effects of stimulation of autonomic nerves on pressure in the corpora cavernosa penis (CCP) were studied in intact rats and in rats in which the pelvic nerves were unilaterally or bilaterally transected. In one animal, an increase in penile pressure was observed after a unilateral pelvic nerve transection. No increase in penile pressure was observed even with bilateral stimulation of the hypogastric nerves. In one animal, the pelvic nerves were stimulated after the injection of the retrograde tracer into the CCP of the contralateral side. A more consistent rise in pressure occurred with stimulation of the hypogastric nerves. However, no increase in penile pressure was observed even with stimulation of the hypogastric nerves. The results suggest that the pelvic nerves may have a role in the control of the penile circulation.


The distributions of pelvic nerves which supply the male rat were defined by using retrogradely transported dyes, injected into the bladder, colon or penis. The majority of pelvic nerves that supply the bladder, colon or penis contain acetylcholinesterase and fibers immunoreactive for vasoactive intestinal polypeptide (VIP). The results suggest that the pelvic nerves may have a role in the control of the bladder, colon or penis. The pelvic nerves may be involved in the control of the bladder, colon or penis. The pelvic nerves may be involved in the control of the bladder, colon or penis.
REORGANIZATION IN CAT PELVIC PLEXUS FOLLOWING PARASEYMPATHETIC-PREGANGLIONIC LESIONS. E. Greenbaum, A.M. Booth, M. Kawatani & W.C. de Groat, Deps. of Pharmacol. & Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Chronic section of the parasympathetic preganglionic innervation to the pelvic plexus results in the evolution of new patterns of pelvic nerve (PN) firing on PN preganglionic activity. Neural firing on PN preganglionic fibers in response to pelvic (PM) or hypogastric (HGN) nerve stimulation. These patterns included: 1) sustained PN discharges evoked by stimulation of the HGN, and 2) increased asynchronous neural activity in PN preganglionic fibers following a tetanic stimulus to the lesioned PN. Conventional in vitro intracellular recording techniques were used to examine the synaptic correlates of these events more than one year after resection of the sacral dorsal and ventral roots in the adult cat. Lucifer Yellow was injected into most cells to examine their morphology.

These recordings revealed: 1) a high percentage of cells without synchronously evoked fEPSPs, 2) fewer inputs to innervated cells that were of abnormally long latency and large amplitude, 3) an evoked asynchronous discharge in the majority of cells, 4) different thresholds for fEPSPs and asynchronous discharge, and 5) abnormal cell morphology. These results confirm significant synaptic reorganization within bladder ganglia of the cat following partial denervation.


Previous studies revealed that leucine-enkephalin (LEUK) was present in sacral preganglionic neurons (SPGN) which innervate the urinary bladder and in varicosities surrounding postganglionic neurons in bladder PSG. The varicosities were eliminated after sacral ventral root transection indicating that they were varicosities of SPGN. Present studies examined the ultrastructural characteristics of LEUK terminals in the PSG. Experiments were conducted in 7 adult cats using standard EM-immunohistochemistry. LEUK was identified only in axons and terminals in PSG. LEUK positive terminals exhibited large dense core vesicles and small clear vesicles. LEUK-ir was primarily associated with the large dense core vesicles. The size of the average size of the large dense core vesicles was 105 nm whereas small clear vesicles averaged 50 nm. LEUK terminals made synaptic contact with the dendrites and soma of the principal neurons. Axo-axonic contacts were identified in few cases. The synapses were of the symmetrical type. These observations suggest that LEUK is a co-transmitter with acetylcholine in the sacral preganglionic outflow of the cat.

AN EXTRACELLULAR STUDY OF THE RESPONSES OF LUMBAR SYMPATHETIC PREGANGLIONIC NEURONES TO NOXIOUS STIMULATION OF THE SKIN IN THE CAT. M.P. Gilbey, J.F.R. Paton* and J.M. Clark*, Department of Physiology, Royal Free Hospital Medical School, Rowland Hill Street, London NW3 2PF, U.K.

Response characteristics of lumbar sympathetic preganglionic neurons (SPN) to noxious stimulation of the skin have been studied previously using single fibre recordings (Janig, W., Rev. Physiol. Biochem. Pharmac., 102, 119-213, 1985). In this study extracellular recordings from SPN have been made as the technique permits cells to be driven pharmacologically. M.P. Gilbey, M.R.S. Perks, J.F.R. Paton and J.M. Clark*. Physiol. Rev., 62, 1989-215, 1985. and therefore inputs to "silent" SPN as well as those having ongoing activity can be studied. Sixty four stimuli have been given to chloralose anaesthetized cats. These were identified antidromically by stimulation of the lumbar chain between L5 and L6 ganglia. Of these, 50% were activated in response to noxious input (heat 50-60°C). 2 ceased to discharge, one had a slightly decreased discharge during the stimulus and the rebound excitation and 3 were unaffected. One neuron having ongoing activity was unaffected by the stimulus. These results show that the discharge of glutamate-activated SPN can be influenced by noxious stimulation of the skin.

Supported by the British M.R.C. and British Heart Foundation*.


We have assessed the effect of inputs that are known to influence spontaneous contractions of the urinary bladder (CUB) on CUB elicited by electrical stimulation of the pontine micturition center (PMC). ES of the proximal cut S2 ventral root (2V, 20 Hz, 0.05 msec) or mechanical stimulation of the region inhibited both spontaneous CUB and CUB elicited by PMC stimulation. However perigenital (PG) stimulation which inhibited spontaneous CUB, facilitated CUB elicited by PMC stimulation. Section of dorsal roots L7 through S3 abolished spontaneous CUB but not the CUB or the relaxation of the external urethral sphincter (EUS) elicited by ES of the PMC (90-100 uA, 0.3 msec, 300 msec trains at 50 Hz). CUB elicited by PMC stimulation in the deafferented animal were not affected by neuromuscular blockade but were abolished by section of the sacral ventral roots. We conclude that the U/EUS synergy during micturition is organized within the CNS independent of sacral afferent input and that recurrent inhibitory, rectal inhibitory and PG facilitatory inputs modulate the descending PMC pathway whereas PG afferent inhibitory input likely acts on the afferent arc of the spinobulbospinal micturition reflex.
nociception. GABA may exert a tonic inhibitory influence on lamina I-II neurons. Similar relationships indicate an excitatory input to lamina I-Π neurons by SP in the control of adrenal blood flow, whereas BMI- and muscimol-evoked changes in CA secretion were assessed from adrenal vein plasma samples by HPLC with EC and fluorometric detection. Catecholamine cells and fibers are also found in IRt but they are more scattered in the epineurial plexus than in IRl. These data suggest that TRH projections to the DMV arise from neurons in the nucleus of the tractus solitarius (nTS) and their apparent target neurons in the rat gut. A. L. Kiechlepfer and M. G. Gershun, Dept. of Anatomy & Cell Biology, Columbia Univ. P&S, New York, NY 10032.

Although the intravenous injection of saline is independently capable of mediating reflexes, the gut receives a CNS innervation from the dorsal motor nucleus of the vagus (DMN). Since the number of efferent vagal fibers is small, it is not clear how the vagus affects enteric activity. The current experiments were undertaken as a partial test of the hypothesis that the CNS innervates a small number of commissural neurons in enteric ganglia. The aim of the present study was to demonstrate the immunoreactivities of VIP, enkephalin, galanin-5, and tyrosine hydroxylase (TH) along with that of PHA-L. Vagal afferents were examined by anterograde labeling of the进入 the nTS-dmnX, against Met5-enkephalin was examined in relation to sensory and motor components of the dorsal vagal complex. The afferent and efferent neurons were identified by anterograde and retrograde transport of horseradish peroxidase applied in a cuff around the proximal and distal ends of the cervical vagus. This was followed by light microscopy, enkephalin-luc immunoreactivity (ELI) was seen as silver grains diffusely distributed throughout the neuropil and in labeled varicose fibers located within the medial nucleus of the solitary tract (m-NTS). Portions of the m-NTS containing ELI also contained extensive anterograde labeling of vagal afferents. Conversely, the motor nuclei of the vagus contained retrogradely labeled perikarya and proximal processes. Motor neurons were surrounded by silver stains of ELI. By electron microscopy, ELI was associated with large dense core vesicles in axon terminals. Terminals containing ELI were detected in both sensory and motor portions of the vagus complex as identified by anterograde labeling of axon terminals or retrograde labeling of perikaryas and dendrites. These results suggest that opioids may be important modulators of both sympathetic and parasympathetic reflexes involving the dorsal vagal complex. (Supported by NIH grants MH00078 and HL11874 (VMP) and INSERM 86024 (LVI)).

Recent investigations have found that the paraventricular area serves as the major afferent to the locus coeruleus (LC) and a key element in the processing of autonomic and visceral signals. We have investigated afferents to LC, we have re-examined this issue as to all the visceral afferents to LC, using recently developed, more sensitive transport techniques.


The central nucleus of the nucleus of the solitary tract (NTS) innervates the visceral and somatic responses associated with various adaptive behaviors, such as fear and the defense reaction. The nucleus of the solitary tract in the left foreplay for cardiovascular afferent fibers, projects to the central nucleus. In this study, the possibility that catecholaminergic neurons in both the ventrolateral medulla and NTS innervate the central nucleus. Central catecholaminergic, the NTS and ventrolateral medulla have each been implicated in the autonomic control of blood pressure. The catecholaminergic projections from the NTS and ventrolateral medulla may convey cardiovascular interoceptive information to the central nucleus. Studies are in progress to the central nucleus from the central nucleus arises from the afferent (C1/C2) and noradrenergic (A1/A2) group of catecholamine neurons within the brainstem. (Supported by Sigma XI grants-in-aid of Research and NIH NS 20041).

527.23 STATIC MUSCULAR CONTRACTION ALTERS THE DISCHARGE FREQUENCY OF NEURONS IN THE CENTRAL NUCLEUS OF THE SOLITARY TRACT. R.M. Bauer*, G.K. Iwamoto and T.D. Walldorf* (Spon. J.A. McMullan) Depts. of Physiology and Vet. Biosciences, University of Illinois, Urbana-Champaign. The areas in the central nervous system which modulate the sympathoexcitatory responses to muscular contraction are not well understood. Recent studies from this laboratory have demonstrated that the reflex arterial pressure response to muscular contraction can be attenuated by bilateral microinjections of a glucose antagonist into the rostral ventral medulla (RVLM) and caudal nucleus (RN). The purpose of this study was to determine if static contraction of hindlimb muscles alters the sympathoexcitatory responses in RVLM. In anesthetized cats, contractions elicited by stimulation of LF and SI central roots evoked increased in arterial pressure, heart rate and minute volume. The majority (70%) of cells (n=50) recorded from within the RVLM (2.0 mm rostral and 3.75-4.5 mm lateral to dura and 4.5-5.5 mm below the dorsal surface of the brainstem) responded to contractions with a sustained 70% or greater increase in firing rate. The firing frequency of neurons recorded from sites dorsal and lateral to the RVLM were not altered significantly during contractions. These findings suggest that the reflex increases in cardiorespiratory activity evoked by muscular contraction involve neurons in the RVLM. (Supported by Am. Heart Assoc.)

527.24 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ANGIOGENINS IN THE AREA POSTERIOR AND VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. J.L. Rapold and S. Frank. Dept. of Physiology, Queen’s University, Kingston, Ontario, Canada.

The area postrema (AP) is a midline circumventricular organ located on the dorsal surface of the caudal medulla in the rat, overlying the nucleus of the solitary tract (NTS). It sends major projections to the parabrachial nucleus (PNB) and contains a high concentration of angiopeptin (AII) receptors. In many species, the AP is thought to centrally mediate AVP (vasopressin) and NA activity in the cardiovascular system. In this study, electrophysiological techniques were used to investigate the effects of systemic AII on neuronal activity in the AP and NTS, and to examine connections between AP and the PBN.

Recordings were obtained from neurons in the AP and NTS of anesthetized male rats and the levels of IL-1 beta and TNF-alpha were measured in the AP. Preparatory activity or baseline activity and the 25% of cells were antidromically identified as projecting to the PBN in the AP and NTS. The remaining cells in the AP and NTS region were orthodromically identified as projecting to the PBN. These findings suggest that systemic AII affects opposing effects on cells in the AP and NTS. Further experiments will elucidate the mechanism of this action and the effects of neurotrophins projecting from the AP to the PBN. Supported by the MRC of Canada.
DISTRIBUTION OF VASOPRESSIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN CANINE PARAVENTRICULAR AND SUPRAVENTRICULAR HYPOTHALAMUS. G.H. Block and H. Villemé. The Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44193.

From initial anatomical studies, vasopressin (VP) and vasoactive intestinal polypeptide (VIP) are co-distributed in the paraventricular (PVN) and supraoptic (SON) nuclei of the rat. In the dog, vasopressinergic neurons are primarily located in the nucleus of the solitary tract (NST) and the parabrachial nucleus (PBN). VP and VIP immunoreactivity are observed in the paraventricular nucleus (PVN) and SON and distributed in the lateral aspect of the posterior magnocellular division. Double-labeled neurons containing VP and VIP have been identified in the posterior magnocellular division, and VP and VIP immunoreactivity has been observed in the paraventricular nucleus and SON. These results suggest that VP and VIP may play a role in the regulation of water balance and fluid homeostasis.


Arginine vasopressin (AVP), released from nerve terminals in the ventral septal area (VSA), acts as an endogenous antidiuretic to control febrile hyperthermia (J. Physiol. 387:163-172). AVP is co-localized with CRF in the paraventricular nucleus (PVN) and SON. AVP and CRF immunoreactive fibers have been observed in the paraventricular nucleus, paraventricular nucleus, and SON. These results suggest that AVP and CRF may play a role in the regulation of water balance and fluid homeostasis.

OLFACTORY INPUT TO AMYGDALOID NEURONS PROJECTING TO THE DORSAI MEDIULLA. M.D. Casella1 and L. Marder2. (SPSR: J. West). Dept. Anat., Univ. of Iowa, Iowa City, IA 52242.

The present results yield evidence for the existence of olfactory inputs to the central amygdaloid nucleus (Ce). The olfactory projections terminate in the medial Ce and are in a position to influence Ce neurons projecting to the medulla. We have therefore examined the post-synaptic relationships of TT terminals in the rat Ce at the ultrastructural level. The results suggest that the Ce neurons may be directly influenced by olfactory inputs.


Electrical stimulation of the infralimbic cortex (ILC) decreases blood pressure and inhibits gastric motility. We examined the efferent projections of ILC using the retrograde tracer PHA-L. Three efferent fiber bundles were observed. (1) A dorsal projection innervated the preoptic and paraventricular nuclei, extended to the dorsal forebrain and the dorsal subiculum. (2) Fibers in a lateral projection extended caudally in the ventromedial portion of the ventral tegmental area to innervate the dorsal piriform and insular cortices. (3) The largest group of labeled fibers extended to the central tegmental field and the parabrachial nucleus. Fibers extended into the medial and lateral portions of the medulla and tegmentum and innervated the spinal trigeminal nucleus, reticular forebrain, nucleus ambiguus and the ventrolateral, medial and commissural portions of the nucleus of the solitary tract. These efferent projections may provide the neuroanatomical substrates mediating the physiological responses observed during electrical stimulation of ILC.
528.1


The adrenal medulla was analyzed with catecholamines (CA) and peptides. While the role of the latter is not understood, it generally is agreed that they are released with the adrenomedullary gland in response to various stimuli. Abundant chromaffin tissue also exists in extraadrenal sites, but the function of these "paraganglia" cells is totally unknown. In order to determine if this tissue responds to stimuli as does the adrenal medulla, animals were subjected to a hypothroid environment and examined adrenal medulla cells of the nerve. Tissue were anatomically mapped with potassium dichromate and then processed for fine structural study. Following injection, 4°C, 3°C reticulate temp., or more severe (10 mins. in 0°C ice bath, 15°C rectal temp.) hypothermic stress, medullary CA granules readily showed changes ranging from partial to total depletion of the dense core usually associated with CA storage. Conversely, even the more severe hypothermia failed to affect the paraganglion granules to the same degree, as the granule core always was present. Occasionally, some granules were swollen and vacuolization was evident within the cytoplasm, nevertheless frank depletion was not noticed. Paraganglion cells may not respond to this stimulus, particularly when one considers that they do not receive nerve endings.

528.2


Maternal Hypoxia (MH) alters pre and postnatal opiate levels in rabbit brainstem (Dev. Neurosci. In Press). We hypothesized that MH would also alter pre- and postnatal opiate levels. 12 pregnant animals were placed in environmental chambers at gestational day E0. Between days E14 and E28, 6 pregnant animals were bled 212 (0.1%) and 40% of the animals breathed 14% O2 (MH). On E28, 3C and 3H animals were delivered by hysterotomy. The remaining animals kindled at term. H was determined on day 1, P3, P7 and P21 and adrenal medullae removed for measurement of opiates and catecholamine levels (NE,DA,E). NE, DA, E content/protein was determined by MH. Opiate results are below (pmol/mg protein ± SD).

AGE: Native (N) Enkephalin: HIC

528.3


The BRB (the P2 cross of SHR x WKY) has a marked sensitivity to tail shock stress and dietary salt intake. Since the adrenal medulla and its anorexigenic properties are thought to play a permissive role in the development and progression of hypertension, we examined adipocyte enkephalins, NA, epinephrine, E, dopamine, DA, and serotonin, 5-HT in BRB given normal (N) or high salt (HS) diets and exposed daily to swimming, tail shock or neither. Our results (KEMEM) are summarized below.

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<th>Diet</th>
<th>E (pg)</th>
<th>DA (pg)</th>
<th>HT (pg)</th>
</tr>
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<tr>
<td>S</td>
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<td>21.93±0.4</td>
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<tr>
<td>T</td>
<td>5.2±0.3</td>
<td>21.93±0.4</td>
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<tr>
<td>NT</td>
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<td>21.93±0.4</td>
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528.4

INCREASED ANGIOTENSIN II RESPONSES ARE NOT REQUIRED FOR POTENTIATION OF ADRENAL CATECHOLAMINE RESPONSES TO REPEATED HEMORRHAGE IN DOGS. J. Lawton*, P. J. d'Almada* and D. S. Com (SPOX: M. Carlson) Dept. of Surgery, St. Francis Hospital, Rhode Island, Providence, RI. 02905.

Potentially adrenomedullary (CA) secretory responses have been described to the secretory responses of A-II in dogs. Angiotensin II (A-II) is an adrenal CA secretory stimulus. In order to determine the role of A-II in the CA secretory response to hemorrhage, we performed hemorrhage (hem) with epinephrine and adrenal vein and femoral artery and vein cannulation. Two days later under pentobarbital anesthesia, dogs were bled 20% (N) or 30% (MH) of their body weight (Evan's blue 0 to 1%). E. No hemorrhage was studied after each hem. CA were measured by HPLC and adrenal secretion rates of A-II in CA responses to hem. Supported by NIH Grant GM-27946.

<table>
<thead>
<tr>
<th>Diet</th>
<th>E (pg/min)</th>
<th>DA (pg/min)</th>
<th>HT (pg/min)</th>
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<tbody>
<tr>
<td>N</td>
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528.5


Hypoglycemia produced by repeated treatment with insulin results in increased adrenomedullary catecholamine (CA) synthesis as evidenced by an induction of tyrosine hydroxylase (TH). The extent of TH induction and CA release in the adrenal medulla was examined in male Sprague-Dawley rats.

Rats were treated with protamine-zinc insulin (Lilly) at 12 hr intervals for 4 days (4-8 U/day, s.c.), causing a two-fold increase in adrenal TH activity. Adrenal CA release was assessed in pithed rats 16-18 h (4°C) after the final insulin injection. Plasma catecholamine concentration and absolute adrenal CA content had returned to normal levels. Electrical stimulation of the thoracolumbar spinal cord (4-32 Hz) caused an increase in plasma epinephrine and plasma norepinephrine that were equivalent in insulin- and saline-treated rats. Simultaneous values were also equal. Insulin had no effect on clearance of epinephrine from the plasma. Similar results were obtained when the adrenal was field stimulated in situ at 8 Hz for 1 hr.

These findings suggest that the induction of TH that results from insulin-induced hypoglycemia is not associated with a change in the basal or neurally mediated adrenal CA release. (Supported by NIH Grant MH-29670 and NSF BNS-8118035)

528.6


Following an insulin-induced hypoglycemic insult, patients demonstrate a peak in plasma chromogranin A (CRA) levels that is not associated with a similar increase in plasma CA. However, when hypoglycemia is induced by 70% hypobaric hypoxia, a CRA peak by about 90 min (Clinical Res. 35:605A, 1987) from the observation of such a peak, we propose there are at least two different types of CRA secreted from the adrenal chromaffin cell. Some secreted access the circulation via different routes. Plasma and lymph were collected under baseline conditions, the adrenal medulla cannulated, and one group of animals placed in the left adrenal medullary vein and thoracic duct, respectively, in both groups. Blood glucose concentration was determined after collection of baseline samples. Blood glucose was then determined in samples taken from the femoral vein and insulin (1 U/kg) was then given after collection of baseline samples. The CRA levels of these animals were collected for an additional 90 min. In three cats, the adrenal glands were isolated from the circulation after the first hemorrhage and collected for an additional 90 min. Free CRA (MW=133-183) levels in adrenal medulla were significantly exceeded those in lymph. Neuropeptide Y (MW=4254) was found primarily in plasma, but was also present in lymph. CRA A (MW=48-58) was detected in approximately equal amounts in plasma and lymph. After the adenals were isolated, CRA levels dropped in plasma. We conclude that larger molecules secreted from the adrenal medulla may enter the circulation via the lymphatic system.

Changes in adrenal medullary and cortical blood flow after hemorrhage (hem) have been described using radioactive microspheres (mcs). To assess changes in intracortical adrenal blood flow, a method was used to measure intracortical distribution of non-radioactive mcs. Injection of different colored fluorescence multiple determinations of blood flow. Penetratatorial anesthetized dogs (n=6) were prepared acutely with left ventricular and arterial catheters for injection and collection of 15 µm mcs, respectively. Adrenal denervation was done unilaterally by cutting the thoracic splanchnic nerve. Injections were made prior to and immediately after 18 ml/kg hem (duration 5.4 min.) and adrenal mcs were removed, fixed and sectioned at 80 µm. Using fluorescence microscopy, mcs counted with fluorescence dye were counted in each section. The number of mcs in the capsular zona glomerulosa (ZG), inner cortex (IC), and the medulla (MED) was determined. Prior to hem, CAP received 10.2%, ZG received 65.4%, IC received 5.9%, and MED received 18.9% of adrenal blood flow. Denervation did not change this distribution. Following hem, in intact glands, the proportion of flow to the MED increased to 35.7%, while proportions to CAP, IC and ZG decreased to 5.6, 35.1, and 3.9%, respectively. Distribution of intracortical blood flow did not change. In denervated glands, changes in all zones after hem were reduced. Since sinusoids in the IC do not contribute significantly to blood flow in the ZG, to test this possibility, 5 and 8 µm mcs were injected. The ratio of IC space to total adrenal mcs increased inversely with sphere diameter, suggesting that blood flow determination with 15 µm mcs underestimates IC flow. These data show that whereas hem increases medullary blood flow in intact glands, removal of noradrenaline causes changes in distribution of intracortical blood flow. These findings support and extend work suggesting that the distribution of adrenal blood flow is influenced by adrenal innervation. Supported by NIH grants DK38391 and DK52631.

PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF THE MEDIAL THALAMUS. J. D. Dunn and I. Calloway*. Dept. of Neuroendocrinology, Rockefeller University, New York, NY 10021

We pursued the possibility that the medial thalamus is involved in adrenocortical function, blood samples obtained prior to and following electrical stimulation of female rats were assessed for corticosterone (Cort B) concentration. Rats were anesthetized with urethane (1.3g/kg), trachealized, instrumented, and positioned in a stereotaxic apparatus. EEG, HR, MAP, and hippocampal EEG were monitored and timed samples (0.2ml) were obtained throughout the experiment. Blood samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100µA, 0.5 µsec off/on, 600 Hz). A change in plasma Cort B was considered different from no change when the average of the 5,10,15 and 30 min. samples deviated by more than 10% from the pre-stimulus level. Stimulation of all subdivisions of the dorsomedial nucleus produced an increase (+21%) in plasma Cort B levels. Stimulation of the parameatal corticostatic nucleus resulted in decreased (-16%) plasma Cort B levels. In contrast no change in Cort B levels were observed following sham stimulation or stimulation of other surrounding sites, including the medial habenular nucleus. Collectively these data support the hypothesis that the medial thalamus contains areas differentially involved in adrenocortical function.

AMPLITUDE MODULATION OF A BURST-LIKE MODE OF CORTISOL SECRETION MISES HIZE TO THE CINCINNATI CORTICOTROPIN RHYTHM IN RATS. D. Velhuis, A. L. Ramamurthi, G. Lizaradre, H. L. Robinson.* Univ of Virginia School of Med, Charlottesville, VA 22908; Dept of Int Med, Veterans Administration Medical Center, Salem, VA 24153.

We examined mechanisms subserving the in vivo circadian rhythm of cortisol in man. Blood samples were drawn at 10 minute intervals for 24 hr in each of 6 men to yield well defined episodic cortisol release profiles. A multiple-parameter decovolution model was applied to describe the number, amplitude, frequency, and phase of all significant underlying cortisol secretory bursts, and simultaneously estimate the endogenous half-life of cortisol. These experiments defined the limited cortisol secretory bursts with a mean interburst interval of 774±0.2 min. The amplitude of secretory bursts was 2.5±0.2 ng/ml (mcs/ml/min) in the absence of light (duration at half-maximal amplitude) only 160±4.1 min. We calculated an in vivo cortisol disappearance half-time of 75.5±9.2 min and production rate of 162±4.1 ng/ml/day (161±4.1 ng/day). Cortisol secretory burst frequency varied 2.2 fold over 24 hr, whereas secretion rate remained constant throughout the day. The circadian rhythm of cortisol secretion can be accounted for by a model of amplitude-modulated burst-like cortisol secretion in the IC as in series with ZG capillaries, the small proportion of mcs in IC may result from trapping of mcs in the ZG. To test this possibility, 5 and 8 µm mcs were injected. The ratio of IC space to total adrenal mcs increased inversely with sphere diameter, suggesting that blood flow determination with 15 µm mcs underestimates IC flow. These data show that whereas hem increases medullary blood flow in intact glands, removal of noradrenaline causes changes in distribution of intracortical blood flow. These findings support and extend work suggesting that the distribution of adrenal blood flow is influenced by adrenal innervation. Supported by NIH grants DK38391 and DK52631.

GLUCOCORTICOID REGULATION IN RESPONSE TO CHRONIC ETHANOL TREATMENT. R. L. Spencer, X. Ximena and B. S. Genn, Lab of Neuroendocrinology, Rockefeller Univ, New York, NY 10021.

The response to an acute dose of ethanol (EtOH) resembles a stress response, characterized by a rapid rise in serum corticosterone (Cort). We examined the effect of chronic (1-3 g/kg, subcutaneous) EtOH treatment on glucocorticoid regulation. In 1 experiment, male Sprague-Dawley rats were given EtOH (2.5-3.0 g/kg, i.p.; 20% v/v) twice per day (9AM and 4PM) for 18 days. The EtOH treated rats did not show any increase in body weight and thymus weight, and increase in adrenal/body weight relative to saline injected controls. These findings are consistent with no change in glucocorticoid receptor (GR) Type I or Type II binding levels in the hippocampus (HC). EtOH treated rats had elevated basal serum Cort levels at 3AM on day 19, but a blunted Cort response to 1 hr of restraint stress relative to control rats. In a second experiment with male Long-Evans rats, the same EtOH treatment paradigm produced a similar pattern of body and tissue weight changes, as well as a significant increase in GR Type I binding in the hippocampus. Type I binding in the hippocampus can be reversed by chronic EtOH treatment. Some of these changes may be adaptive and help to diminish the acute EtOH response to ethanol. Supported by MH41256 and AA05256.
VIP BOUNDING AND STEROIDGENIC EFFECTS IN THE ADRENAL CORTEX.

N. A. Hughes and M. A. Goldsmith. Department of Cell and Structural Biology, University of Illinois, Urbana, IL 61801.

VIP-immunoreactive fibers innervate the capsule and zona glomerulosa regions of the rat adrenal cortex. Previous studies demonstrated direct VIP stimulation of steroidogenesis in zona glomerulosa tissue preparations perfused in vitro (Endocrinology 122(5), 1988). The present studies were undertaken to determine whether VIP binding sites exist in the zona glomerulosa.

Specific activity of 122H-VIP binding in this region was demonstrated; binding of 0.75 nM [3H]-VIP was inhibited (65%) in the presence of unlabelled VIP (7.5 µM), which was unaffected by ACTH (7.5 µM), and was decreased 20% by 7.5 µM angiotensin II. VIP stimulation of aldosterone, but not corticosterone, was enhanced by the humoral secretagogues ACTH/aldosterone secretion stimulated by 10^{-5} M VIP in vitro was enhanced 70-80% by concomitant stimulation with subthreshold concentrations of ACTH (10^{-11} or 10^{-10} M). VIP-stimulated steroidogenesis was not affected by the presence of 10^{-9} or 10^{-8} M angiotensin II.

Supported by NIH 50678.


During states of extracellular fluid and body sodium loss the mineralocorticoids (MIN) are elevated in the body and play a fundamental role in the retention and redistribution of body sodium. The hormone also generates a hunger for salt in the rat. The glucocorticoids (GLU) are to a much less degree elevated during body sodium depletion and act natriuretic. While both hormones compete for the same receptor sites in several limbic brain regions, and there is a far greater number of GLU than MIN sites, these sites also show preferential binding. A MIN target is the preoptic area and hypothalamus: a GLU target is the hippocampus. In the present study we confirm and extend that MIN induced salt appetite in addition to body sodium depletion induced salt appetite is further enhanced when combined with GLU. And that corticosterone administration increases the level of aldosterone binding to preoptic-hypothalamic, but not to hippocampal MIN receptors. One hypothesis is that elevating MIN receptors by GLU action in preoptic hypothalamic tissue contributes to the natrioregic actions of the mineralocorticoids.

Supported by NIMH 50678.


We report here on the developemental changes in 3H RU 28362 (glucocorticoid receptor) and 3H aldosterone (mineralocorticoid receptor) binding capacity in soluble fractions prepared from hippocampal tissue. 3H RU 28362 binding was low on Day 3 of life (30% of that observed in adults) and increased towards adult values during the second and third weeks of life, a pattern virtually identical to that previously reported for dexamethasone binding. In contrast, aldosterone binding on Day 3 of life was only slightly less than that observed in adults and reached adult values by Day 7 of life. Postnatal handling, which has been shown to increase dexamethasone binding in hippocampus, resulted in a significant increase in 3H RU 28362 binding capacity in hippocampus, but had no effect of aldosterone binding. These results confirm that handling selectively influences the glucocorticoid receptor system in hippocampus. (Supported by MRC grants to MM).

Supported by NIH grants R01-RR-05410-26.

Characterization of 3H RU 28362 BOUNDING TO TYPE II GLUCOCORTICOID RECEPTORS IN NEURAL, Lymphoid, and Pituitary tissue. Yun-Chia Chou*, William G. Luttge, and Colin Sumners (SPON: W.A. Friedman). Dept. of Neuroscience & Physiology, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610.

The purpose of this investigation was to study the properties of glucocorticoid-type II receptors in brain cell cultures. Cytosol was prepared from primary cultures of neurons of glia and incubated with [3H]Dexamethasone at 0°C. Free steroid was removed afterwards by gel filtration on G-25 columns. At a steroid concentration of 20 nM, [3H]DEX-Type II receptor binding in cytosol was found to be equally effective in suppressing the increases in the binding capacity of both of these receptors. This equilibrates with the anti-adrenal site of DEX vs. CORT for Type I receptors, but it is not compatible with the much lower endogenous Type II receptor levels when compared to Incubations in PDHS. Switching cells from neuronal and glial primary cell cultures should be an important model system for further research on the molecular mechanisms of glucocorticoid actions in brain.

(Supported by NIH grants HL-36645 and NS-24404.)
NEURAL CONTROL OF ADRENAL FUNCTION

This study was designed to identify effects of corticosterone on hippocampal neuroarchitecture using quantitative electron microscopy. Sections from regions CA3B, CA1, and dentate gyrus were examined in unstressed controls and cort-treated rats (20 mg/kg in saline o.d./i.d. x 3 days) each dose produces cort levels in the upper physiologic range for 20 hrs. The density and surface area of Golgi and the number of cisternae increased (38-50%) in neuronal perikarya (p<0.001, ANOVA). In addition, the surface area of rough endoplasmic reticulum and numbers of cisternae increased (38-50%) in neuronal perikarya (p<0.001, ANOVA). Densities of mitochondria, multivesicular bodies, and lysosomes in perikarya were unaffected. These data suggest that high doses of cort on not only increase protein synthesis, but also may alter subsequent processing by the Golgi and they agree with studies of Finch et al. (1987) showing a similar time course of cort induction of an array of hippocampal mRNAs. Supported by NIH HD 19431, NIH AG-06531, and FRSO.

528.19 
ACUTE EFFECTS OF CORTICOSTERONE ON RAT HIPPOCAMPAL PERIKARYA. E. Antic, L. Tagliati, P. Antinori, and G. Tagliati, Department Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada, H3A 1A; Department of Biology, Stanford University, Stanford, CA, 94305.

528.20 
ACTIVATION OF SYNAPTIC PLASMA MEMBRANE CALCIUM-ATPASE BY GLUCOCORTICOIDS. P. Antinori and E. Antic, Department Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada, H3A 1A; Department of Biology, Stanford University, Stanford, CA, 94305.

The results of the experiments of the developing molecular layer (ML) and heavy label over the developing molecular layer (ML) and (35S antisense RNA) studies of developing mouse cerebella show that cDNA clone, pMuBr3, which, in adult cerebellum, preferentially hybridizes to Purkinje cell soma in situ. In situ hybridization studies of the rat fetal cerebellum cDNA library cloned in lambda-probe. Thus, this method can be used to screen mRNAs of low abundance. Most of the fetal cerebellar mRNAs were expressed at similar levels in the adult rat cerebellum. 20% were reduced several fold and 2X were increased in development. Five mRNAs were highly enriched in fetal cerebellum. Supported by NIH HD14886 and the Leland Beach Foundation.

529.1 
GNE EXPRESSION CHANGES IN DEVELOPING AND MUTANT CEREBELLUM. A. Mackersie, R. Neiss, A. Pflummoth-Signier, A. C. Wilson. Department of Biologics and Res, NYS Dept of Health, Albany, N.Y. 12201 & Research Institute, Scripps Clinic, La Jolla, CA, 92037.

529.2 

529.3 
GNE EXPRESSION IN RAT CEREBELLAR DEVELOPMENT. G. C. Lit and M. E. Harrison-Toro. Dept of Biochemistry and Neurology, UT Southwestern, Dallas, TX 75235.

529.4 
DEVELOPMENTAL REGULATION OF GAP-43 mRNA. C. G. Mcintyre, D. G. Davis, and D. G. Mcintyre. Medical Products Dept., E. I. du Pont Company, Wilmington, DE, 19898 and Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

We have examined the temporal expression of individual cerebellar g99kDa by plaque differential screening and by Southern analysis of clones from a 20 day rat fetal cerebellar cDNA library cloned in Lambda-ZAP. Seven hundred plates were screened by plaque differential screening to cDNA probes for fetal and adult cerebellar mRNAs; 8 plates were selected for further analysis. Southern hybridization confirmed that four of these contained sequences enriched in fetal cerebellum. Northern analysis showed that two mRNAs, 0.7 and 1.8 kb in size, were expressed at high abundance in the developing cerebellar cortex. The mRNAs were present in adult tissues, 30-60 fold reduced in adult cortex, and were not detected in adult cerebellum, liver or kidney. In a 3 slice set of experiments, cDNA was isolated from 500 individual plaque (pooled into 150 groups) using our modified rapid mini-isolation method. Southern analysis of the restricted DNA showed that >90% of the inserts were detected by the fetal probe. Thus, this method can be used to screen mRNAs of low abundance. Most of the fetal cerebellar mRNAs were expressed at similar levels in the adult rat cerebellum. 20% were reduced several fold and 2X were increased in development. Five mRNAs were highly enriched in fetal cerebellum. Supported by NIH HD14886 and the Leland Beach Foundation.

529.5 
MESSAGE RN A REGULATION V
mRNA. These data suggest that neurons of the hippocampus, mRNA for Al. Binding was diffuse and of low intensity in NKA, which occurs widely in brain and ion transporting density over the cell bodies of the dentate granule layers of entorhinal cortex also possessed high levels of their electrical activity. Three day old Sprague-Dawley rats were incubated with H-uridine (0.3mCi/ml) for 1 h and 6 hours following labeling. All slices were embedded in plastic and sections of kidney RNA on Northern blots (single band, 4.5 kb) and with sections of in situ hybridization. To identify the types of neuronal and glial cells which are expressed in response the expression of hsp70 genes we have now carried out in situ hybridization using plastic (methacrylate) embedded tissue (sections at three micronic). This permits increased cellular resolution. The patterns of expression of hsp70 genes have been analyzed in the retina, cerebellum, hippocampus and neocortex of control and hyperthermic rabbits using labeled antisense RNA probes (length 255 nucleotides) transcribed from a mouse hsp70 genomic clone (Perry and Moran, Gene, 1987 51: 225-234). Constitutive expression of an hsp70 gene was noted in several neuronal cell types. Induction of hsp70 mRNA was dramatic in glial cells in fibre tracts throughout the brain and was also apparent in several neuronal cell populations. (Supported by grants from the Medical Research Council of Canada),

**DISTRIBUTION OF mRNA FOR THE A1 ISOFORM OF Na*KATPase AND \( ^{3}H \)-GUANABIN IN HUMAN HIPPOCAMPUS.**

Na*KATPase (NKA) is an ubiquitous enzyme which regulates cell membrane potential and volume. Three isoforms exist, the products of separate genes, which appear to have distinctive biochemical properties and anatomic distribution. We used human hippocampal cDNA to compare the distribution of mRNA for the A1 isoform of NKA, which occurs widely in brain and ion transporting tissues. The A1 encoded protein is a 490 amino acid polypeptide, with an apparent molecular weight of 55,000. The expression of this protein was investigated by \( ^{3}H \)-guanabain autoradiography. By in situ hybridization histochemistry performed on surgical specimens of hippocampal region, we find that A1 mRNA was most abundant at highest density over the cell bodies of the dentate granule cells. Neurons in the CA fields, subiculum and deep layers of entorhinal cortex also contain high levels of mRNA for A1. Binding was diffuse and of low intensity in the outer 2/3 and absent in the inner 1/3 of the dentate molecular layer as well as in the ipsilateral entorhinal cortex. Specific guanabain binding was more widely and evenly distributed over the same regions labelled for mRNA, and extended into layer I of cortex. Regional density in guanabain binding was much less marked than for mRNA. These data suggest that neurons of the hippocampus, especially dentate granule cells, have a high levels of NKA compared to surrounding glia, presumably related to their electrical activity.

**LOCALIZATION OF Na*K-ATPase \( \alpha \)-SUBUNIT mRNA AND POLYPEPTIDE IN MOUSE KIDNEY, CEREBELLUM, AND RETINA.**

A mouse encoding mouse Na*K-ATPase alpha-subunit was isolated from a mouse brain lambda gtl 11 cDNA library using antiserum to bovine and mouse brain alpha (combined a1 and a2) subunit. Subsequent to the sequence of this clone was found to be most homologous to that of the rat brain alpha, but not the a2 or a3 isoforms. The \( ^{3}H \)Na*K-ATPase immune probe derived from the cDNA insert, but not the sense strand, hybridized strongly with brain and kidney RNA on Northern blots (single band, 4.5 kb) and with sections of kidney and retina. Specificity for \( \alpha \)-subunit mRNA was further indicated by comparing in situ hybridization of \( ^{3}S \)-riboprobe with immunostaining of a-subunit. In kidney, mRNA and \( \alpha \)-subunit showed similar differential tubular distributions (distal > proximal > thick segments). In retina, inner segments of photoreceptors, the inner nuclear layer, and ganglion cell soma were strongly reactive with both probes. a-subunit, but not mRNA, was prominently expressed in plexiform layers and optic fibers. In cerebellar cortex, \( \alpha \)-subunit and mRNA were localized in basket and granule cell soma. Basal regions and glomeruli, which contain synaptic input from these cells, were strongly reactive for a-subunit. Although Purkinje cell soma expressed abundant message, the somal membrane and emerging dendrite exhibited little, if any, polypeptide, suggesting transport of \( \alpha \)-subunit to more distant sites. These results suggest that co-localization of polypeptide and mRNA will be useful in mapping expression and developmental regulation of Na*K-ATPase at the cellular level. Supported by NIH grants NS15935 and SPM0DK20572.
The role of neuron-glial interactions in the regulation of expression of glial-specific genes is not well understood. Recent data indicate that the expression of glial-specific genes is regulated in response to growth factors and other extracellular signals. The regulation of these genes occurs at both the transcriptional and posttranscriptional levels. The transcriptional regulation of glial-specific genes is often mediated by transcription factors like STAT5, which are activated in response to growth factors. Posttranscriptional regulation involves the alternative splicing of pre-mRNA transcripts, leading to the production of different mRNA species.

Glial fibrillary acidic protein (GFAP) is a marker of reactive gliosis and is expressed in astrocytes in response to injury. The expression of GFAP is increased in response to various stimuli, including cytokines, growth factors, and mechanical stress. The regulation of GFAP expression is important in the repair of damaged neural tissue and in the pathogenesis of various neurological diseases.

The study of the potential modulation of the brain glucose transporter mRNA levels in primary cultures of rat neuronal and glial cells provides valuable insights into the regulation of glucose transport in the brain. The expression of glucose transporter mRNA is tightly regulated, and changes in its expression can have significant implications for brain function and disease. The data presented in this study suggest that the regulation of glucose transporter mRNA levels is mediated by both transcriptional and posttranscriptional mechanisms.
946 RNA: A BRAIN RNA REGULATED BY THYROID HORMONE ACROSS SPECIES AND TISSUES. J.R. Stein, Dept. of Neurology and Psych., Univ. of Md., Baltimore, MD 21205.

The gene expression of the adult rat and mouse brain is sensitive to thyroid hormones. For example, in the rat, the total mRNAs of adult rat brain may be altered by change in thyroid status (Stein, 1988, In press). One of these, S100 RNA (Stein, 1987), in the transition from the ET to NT states: 1) Increased in rat liver and total brain (TB); 2) Decreased in mouse cerebral cortex (CC); and 3) Unchanged in mouse liver on northern gel hybridization (NGH). This demonstrated that thyroid hormone (TH) responsiveness may be related to similar thiolsensitive proteins across species and activities in different tissues within a species. However, the direction of the abundance change in the ET to NT transition in TB and CC may be controlled by other undefined factors. To start to define these factors, we have utilized DNA sequence analysis of the S100 RNA, other TH-regulated RNAs as well as in situ hybridization (ISH) to adult rat and mouse brain. The S100 RNA was converted in double stranded form by modified dideoxy sequencing using Sequenase (US Biological). Because of the secondary structure of the purified S100 RNA, the northern DNA was subcloned into M13 and resubjected by standard dideoxy sequencing. The 350 bp insert was a unique RNA based on the sequence analysis. The initial search of the Gene Bank revealed no similarities with any known protein. On NGH, 946 RNA was more abundant in CC and cerebellum than in other regions. In this brain from a 48 hour ET and NT rodent brain will be reported. (Supported by NIMH #31027 and USF #3177 ET).
Varioys Effects of α-Bungarotoxin and Pernichthysotoxin-1327


The ventral segmental area (VTA) is a brain region which has been demonstrated to mediate at least a portion of the rewarding properties of drugs of abuse. Nicotine is one of the most prevalent agents of abuse in American society. While the effects of nicotine on the neurons of the VTA have been studied in vivo, these studies have been restricted by the limitations of in vivo recording techniques. We have developed a brain slice preparation of the VTA in order to study the effects of known concentrations of agents on neurons in this area. Brain slice containing the VTA were prepared from Sprague-Dawley rats, and superfused at 2 ml/min. The cells that were studied with intracellular and extracellular recording electrodes with electrophysiological properties similar to those of dopamine-containing cells recorded in vivo and in vitro.

Acetylcholine (ACH, 1-100 mM) and carbachol (10-100 M) produced large increases in the spontaneous firing rate of neurons of the VTA. Addition of physostigmine decreased the effective concentration range of ACh to 30-300 M. Nicotine (1-100 M) produced depolarization and an increase in firing rate. Nicotine-induced excitation was completely blocked by the addition of hexamethonium (HEX, 500 M) to the bathing medium. In the presence of 500 M HEX, ACh still evoked an increase in spontaneous firing rate. This ACh-induced excitation was blocked by the addition of atropine sulfate (100-250 M) to the superfuse. These data indicate that both nicotinic and muscarinic receptor activation produce excitation of VTA neurons.
ACETYLCHELONE: RECEPTORS AND CHOLINE UPTAKE

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without effect. While BGT competes for some 125I-NBT binding, it is thought that autoradiographic localization of nicotinic receptors in striatum, suggesting that NBT is a nicotinic antagonist in CNS tissue. Therefore, recently demonstrated that NBT blocks nicotine-stimulated dopamine release in rat

rus multicinctus along with differences somewhat from that reported using other methodologies, supporting the idea that neuronal nicotinic receptors are different in the chicken midbrain. Described the localization of choline acetyltransferase

in the chicken midbrain (Sorenson, E.M., et al., Soc. Neurosci. Abstr. 13:567, 1987). In the same animal, we have observed at least three categories of putative nicotinic receptors using 125I–kappa-bungarotox in and 125I–alpha-bungarotoxin, and for the stratum griseum et fibrosum superficiale (SGFS) in the optic tectum. Although 125I–nicotinic binding to lesser regions than the snake toxins, preliminary results indicate that nuclei which are labeled with 125I–nicotine are also identified by 125I–NBT. While 125I–nicotinic binding patterns are similar, the layer f of the SGFS contain binding sites for all three ligands. (Supported by NIH Grant NS17574 to V.A.C.)

500 ng/ml) did not affect the inward current. The data suggest that a pertussis toxin-sensitive G-protein is involved in the cholinergic activation of the inward current at resting potential. Supported by a NIH Grant NS 26111.

ACETYLCHELINE MODULATES RESTING I-CURRENT THROUGH A PERTUSSIS TOXIN-RESISTANT G-PROTEIN IN HIPPOCAMPAL NEURONS. L.D. Brown, S. Nakanishi and E. Banting. Dept. of Biol. Sci. and Dept. of Pharmacology and Physiol. and Biophys., Purdue University, West Lafayette, IN 47907, USA.

The purpose of the present experiments was to determine if cholinergic excitation produced by a decrease in K-conductance through muscarinic receptors is mediated by a G-protein. We used primary cul tures of hippocampal neurons from rat or chick embryos to test this hypothesis. In the gonadotrophin-releasing hormone (GnRH) system, the inward current was inhibited by atropine (1-2 μM). When the internal solution was replaced by GTPγS (200 μM), a hydrolysis-resistant GTP analogue, the inward current decreased to baseline. This GTPγS-sensitive current is the inward current and is mediated by a pertussis toxin-sensitive G-protein. In contrast, the inward current is not affected by pertussis toxin (500 μM) or by pertussis toxin-resistant GTP. Similarly, this experiment indicates that a pertussis toxin-sensitive G-protein is involved in the cholinergic activation of the inward current at resting potential. Supported by a NIH Grant NS 26111.

We now report the isolation of two new kappa-neurotoxins from the venom of Bungarus multicinctus. Kappa-bungarotoxin and kappa-bungarotoxin exhibit strong sequence homology with Kappa-bungarotoxin. Their pharmacological effects confirm their membership in this new class of snake venom neurotoxins. All four kappa-neurotoxins lack a tryptophanyl residue which is invariant in the structurally similar α-neurotoxins. This and several other features distinguish kappa-neurotoxins from α-neurotoxins, and provide clues to the structural differences between the ligand binding sites regions of neuronal and muscle nicotinic receptors. (Supported by NSF INT-8518395 and NIH-NS15754 to V.A.C.)

REPEATED ADMINISTRATION OF NICOTINE RESULTS IN LONG-TERM DECREASE IN Prolactin RELEASE BY ACUTE NICOTINE IN RATS. B.A. Giblin *, M.D. Lumpkin *, and K.J. Kellar1. Depts. of Pharmacology and Physiology and Biophysics, Georgetown University Medical Center, Washington, D.C., 20007.

Nicotine increases prolactin in prolonged administrations, which peak 6 to 8 min after injection and return to control levels within 30 min. The nicotine-induced increase in plasma prolactin is time- and dose-dependent, is blocked by mecamylamine, and appears to be mediated by receptors in the brain. As previously reported by Sharp and Beyer (J. Pharmacol. Exp. Ther. 230:1846, 1984), when nicotine is injected a second time, 60 min after the first injection, the prolactin response is very much diminished or absent. This is consistent with rapid and prolonged desensitization of the nicotinic receptors mediating prolactin release following the first injection. When nicotine is injected a second time, 60 min after the first injection, the prolactin response to an acute injection of nicotine is absent 2, 4, 6, and 8 days after the last of the chronic injections. This apparent inactivation of the prolactin response to nicotine is present when nicotine receptor recognition sites are increased in the hypothalamus. These results are consistent with the hypothesis that chronic administration of nicotine results in long-term inactivation of nicotinic receptors and that this long-term inactivation is a major mechanism of the nicotinic receptor recognition sites.

Several lines of evidence indicate that muscarinic receptors (mAChR) are heterogeneous. In CNS tissues, activation of mAChR by muscarinic agonists reduces calcium and increases impulsive-dependent acetylecholine (ACH) release from nerve terminals. In the studies described here, we have attempted to ascertain whether inhibition of adenylate cyclase underlies mAChR-mediated inhibition of electrically-evoked [*H]ACH release from rat hippocampus. The muscarinic agonist carbachol caused a concentration-dependent (EC50=100nM) atropine-sensitive decrease in evoked [*H]ACH release up to a maximum of 80% inhibition. The relative potencies of several muscarinic antagonists in reversing carbachol's effect (atropine, N-methyl atropine > scopolamine > pyrilamine) were consistent with an mAChR-mediated response. In the presence of forskolin (10nM) or N-bromo-adenosine 3'-5'-cyclic monophosphate (300nM), electrically-evoked [*H]ACH release was enhanced by 40-50% but the inhibitory effect of carbachol was unchanged. In addition, pretreatment of slices with N-ethylmaleimide markedly reduced carbachol inhibition of adenylate cyclase in hippocampal membranes but did not significantly alter carbachol inhibition of [*H]ACH release. Taken together, these results indicate that inhibition of adenylate cyclase does not underlie mAChR-mediated inhibition of ACH release.


BM-5, an oxotremorine analogue, has been suggested to be a partial muscarinic agonist with tissue specific agonist or antagonist activity. It is thought to act as a ligand in tissues with large receptor reserve and antagonist-like activity in tissues with little receptor reserve. Because of this BM-5 may be useful as a tool in defining tissues with more or less receptor reserve. We have used this adrenergic in studying the agonist- and antagonist-like activity in the peripheral and central nervous system. BM-5 exhibits partial agonist-like properties in vivo. It possesses high affinity for both muscarinic agonists and antagonist binding sites in the rat cerebral cortex, does not alter K+-stimulated release of acetylcholine (ACH) but does block agonist-induced decreases in ACH release from brain slices. These effects are similar to those seen with muscarinic antagonists.

BM-5 has agonist- and antagonist-like properties in vivo. It increases intestinal transit and motility and reverses scopolamine-induced swimming inhibition activity much like a muscarinic agonist, however, BM-5 impairs the ability of C57BL/10 mice to perform a water-maze task in a manner similar to muscarinic antagonists.

Thus, the relative agonist/antagonist properties of BM-5 are governed by the response being measured and may be determined by intrinsic receptor reserves of the target tissue. The therapeutic utility of this compound, however, is questionable since it acts as a muscarinic agonist in the periphery inducing unwanted effects and acts as an antagonist in the central nervous system to impair cognitive performance.


Our previous research (Lai et al., J. Neurochem. 48: 40, 1987; Pharmac. Biochem. Behav. 27:635, 1987) showed that acute (45 min) exposure to circularly polarized, pulsed C450-MHz microwaves at power density of 1.0 mW/cm² (whole body specific absorption rate of 0.6 W/kg) affected cholinergic activity in the brain of the rat. Sodium-dependent high-affinity choline uptake was decreased in the frontal cortex and hippocampus after the exposure. In the present experiment, rats were studied in the brain at 24 h after the last exposure session. There was a significant increase in the concentration of [*H]choline binding sites (p<.005) in the hippocampus of the microwave-exposed rats as compared to that of sham-exposed controls. No significant change in receptor sites was observed in the frontal cortex, striatum, and hypothalamus.


Systemic administration of pilocarpine (30 mg/kg sc) to rats pretreated twenty-four hours earlier with lithium (3.0 mg/kg sc) results in prolonged seizures and acute cytological changes in various brain regions. Muscarinic cholinergic receptor activation is presumed to play a role in the initiation of seizure activity since pretreatment with atropine prevents both the seizures and related brain damage. The brain damage resembles that associated with persistent seizures induced by various other methods and is independent from the excitoxic type of neuronal degeneration that glutamate (Glu) is known to cause. To further evaluate the relative involvement of glutamergic and cholinergic mechanisms in this seizure-brain damage syndrome, we have studied TH-G1b and TH-Glutamate binding by receptor autoradiography in rat brain at various intervals from 4 hrs to 8 weeks after a 3 hr episode of persistent seizures induced by LiPil and treated with nimodipine (Nimodipine) as follows: both TH-Glu and TH-G1b binding were uniformly reduced in early time intervals (up to 2 weeks) in nearly every brain region evaluated, including regions that do not display brain damage. The reductions in TH-Glu and TH-G1b binding were already evident at 4 hrs and became maximal in the 1st to 2 week period then gradually returned toward control levels in the 2-8 wk interval. This pattern was seen in all regions except those known to sustain permanent brain damage; in such regions, binding of both ligands remained severely depressed in the 1-3 wk interval and recovered only moderately in the 4-8 wk interval. In two regions (substantia nigra and nucleus accumbens) a substantial reduction in binding of both ligands at 4-9 hr progressively converted to a significant increase in binding at 4-8 weeks. Based on Scatchard analysis, the decrease in binding for both TH-Glu and TH-G1b could be accounted for by a reduction in Bmax. This was accompanied by a decrease in Kd, for both ligands. These fings are limited in assistance in clarifying the relative importance of cholinergic vs glutamnergic mechanisms in the LiPil seizure-brain damage syndrome since changes in both systems were in the same direction, displayed a similar time course and were of similar magnitude. Supported in part by US Army Contract DAMD17-96-C-6010, ES 0870 and Career Scientist Award NH 38894 (JWO).


Activation of brain cholinergic receptors results in a natriuretic and kaliuretic response. This effect seems to be mediated by both nicotinic and muscarinic receptors. Purpose of this study was to determine whether a depletion of brain acetylcholine stores is able to influence diuresis, natriuresis and kaliuresis. Adult male wistar rats with a chronically implanted intracerebroventricular (ICV) cannula were pretreated with hemicholinium-3 (HC; 10μg/10μl ICV) in 10 different doses. At different time after the icv pretreatment the animals were received 40ml/kg saline by gavage and placed in individual metabolic cages. Urine samples were collected every 2 hours for 6 hours and potassium and sodium excretion were determined. The ICV injection of HC produced a time dependent inhibition of the urinary output with the maximal effect occurring between 60-90 min pretreatment time. Furthermore, ICV HC produced a significant decrease in potassium (0.49±0.02 vs. 0.34±0.01 mg/100 g b.w./6 h) and sodium urinary excretion (1.34±0.08 vs. 0.57±0.05 mEq/100 g b.w./6 h).
POSSIBLE INVOLVEMENT OF CALCIUM AND CALMODULIN IN THE REGULATION OF NASCENT (3H[HCH-3]) BINDING AND HIGH-AFFINITY CHOLINE UPTAKE IN RAT BRAIN. M.D. Sallareli, C.J. Clingroth, K.Yamada, and J.T. Couye. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The sodium-dependent high-affinity choline uptake (SDHACU) system has been regarded as the rate-limiting step in acetylcholine synthesis. Calcium (Ca2+) has been implicated as an essential activator of the SDHACU by many, but not all, investigators. In the present study, we have assessed the involvement of Ca2+ and calmodulin in the regulation of SDHACU through the use of the specific binding of [3H]HCH-3, a potent and selective inhibitor of [3H]choline uptake. Potassium depolarization of striatal, cortical, or hippocampal slices resulted in a significant increase in specific [3H]HCH-3 binding when compared to control slices incubated in normal Krebs buffer containing 1.2 mM Ca2+. The changes in specific [3H]HCH-3 binding induced by potassium depolarization in vitro, hippocampal, but not striatal, slices were significantly reduced when Ca2+ was removed from the incubation medium. Potassium depolarization-induced activation of [3H]choline uptake in striatal slices was also affected by removal of Ca2+ from the incubation medium. In addition, a dose-dependent inhibition of potassium-stimulated [3H]HCH-3 binding was observed when striatal slices were incubated in the presence of three inhibitors of calmodulin, inophenox (INO) = 20 µM, W-7 (HC= 40 µM), and W-5; their potencies for the inhibition of potassium-stimulated activation of [3H]HCH-3 binding are consistent with calmodulin inhibition. Finally, incubation of striatal slices in the presence of the Ca2+ ionophore A23187 (20 µM) produced a marked increase in specific [3H]HCH-3 binding, even when Ca2+ was removed from the incubation medium. These studies demonstrate regional differences in the regulation of [3H]HCH-3 binding by extracellular Ca2+, and suggest the involvement of calmodulin and intracellular Ca2+ in the regulation of SDHACU in vivo.

BIS THIAZOLIUM CATIONS: A NOVEL CLASS OF POTENT CHOLINERGIC AGENTS FOR STUDYING NEUROLOGICAL DISEASE. V. Balasubramanian, M.D. Saltarelli, J. Coyle and H. Wagner. Division of Nuclear Medicine, Johns Hopkins Medical School, Baltimore, MD 21205. Although Hemicholinium-3 is a highly selective potential inhibitor of the choline uptake carrier, its ability to cross the blood-brain barrier limits its usefulness for studies in vivo. To circumvent this, we have synthesized and studied several thiazolium cations as potential cholinergic agents. Since these cations can be reduced to the neutral dihydro analogs, we envisioned the use of the specific binding of [3H]Hemicholinium-3, a potent and selective inhibitor of the sodium-dependent high-affinity choline transporter, as an indicator of cholinergic component and function. Among these, the bis thiazolium cation derived from 4-nethyl-5-[(2-hydroxyethyl)]-thiazole and 4,4'-bis-bromocarbonyl biphthal is a potent competitive inhibitor of choline uptake (K1=10-16-10-17M). Model studies show that these thiazolium cations can be reduced to the corresponding dihydro analogs which, as expected, showed a propensity to undergo reoxidation to parent cations. These results suggest that bis thiazolium cations may be useful ligands for the study of cholinergic structure and function.


A series of carbamyl aprophen analogs were synthesized with carbamyl substitutions at the para position of the phenyl rings to determine whether such compounds would display binary drug functions with therapeutic characteristics of aprophen as well as the ability to mask cholinesterases. These compounds carbamylate human butyrylcholinesterase with similar inhibitory properties to aprophen and also inhibited human serum butyrylcholinesterase in vitro. These results are similar to that of aprophen in terms of its inhibitory effect on the acetylcholine-induced contraction of guinea pig ileum. Although these results with aprophen were similar to that of aprophen in terms of its inhibitory effect on the acetylcholine-induced contraction of guinea pig ileum, the use of aprophen as an inhibitor of [3H]inositol phosphate (IP) formation in bovine brain membranes, the dimethylcarbamyl substituted analogs were inactive in vitro. The lack of activity in vitro, but the inhibition of [3H]inositol binding to [3H]HCH-3 binding in the presence of cyclopiazonic acid (CPA), is similar to that of aprophen in terms of its inhibitory effect on the acetylcholine-induced contraction of guinea pig ileum. Although these results with aprophen were similar to that of aprophen in terms of its inhibitory effect on the acetylcholine-induced contraction of guinea pig ileum, the use of aprophen as an inhibitor of [3H]inositol phosphate (IP) formation in bovine brain membranes, the dimethylcarbamyl substituted analogs were inactive in vitro. The lack of activity in vitro, but the inhibition of [3H]inositol binding to membranes with phospholipase A2 (PLA2) resulted in a significant increase in the specific binding of [3H]HCH-3 to membranes incubated in the presence of 1.2 mM Ca2+. The changes in specific [3H]HCH-3 binding induced by potassium depolarization in vitro, hippocampal, but not striatal, slices were significantly reduced when Ca2+ was removed from the incubation medium. Potassium depolarization-induced activation of [3H]choline uptake in striatal slices was also affected by removal of Ca2+ from the incubation medium. In addition, a dose-dependent inhibition of potassium-stimulated [3H]HCH-3 binding was observed when striatal slices were incubated in the presence of three inhibitors of calmodulin, inophenox (INO) = 20 µM, W-7 (HC= 40 µM), and W-5; their potencies for the inhibition of potassium-stimulated activation of [3H]HCH-3 binding are consistent with calmodulin inhibition. Finally, incubation of striatal slices in the presence of the Ca2+ ionophore A23187 (20 µM) produced a marked increase in specific [3H]HCH-3 binding, even when Ca2+ was removed from the incubation medium. These studies demonstrate regional differences in the regulation of [3H]HCH-3 binding by extracellular Ca2+, and suggest the involvement of calmodulin and intracellular Ca2+ in the regulation of SDHACU in vivo.

THE EFFECT OF AGING ON MUSCARINIC-STIMULATED PHOSPHATIDYLINOSITOL (PI) TURNOVER IN FISHER 344 RATS. B.D. Schwarz, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Receptor number and function of cholinergic neurotransmitters, including ACh, have been shown to be altered during aging and in neurodegenerative disorders associated with aging (e.g. Alzheimer's disease). Recently, we reported that cortical M1 muscarinic receptors were significantly decreased in aged Fisher 344 rats (J. Cell. Biochem. 11D: 198, 1987). Since it has been suggested that M1 receptors are functionally coupled to phosphatidylinositol hydrolysis, the present study examined whether these age-related changes in PI turnover using the method of van den Berg et al. (1986). PI turnover was measured in cortical slices from young (3-5mo) and aged (22-24m) male Fisher 344 rats. In young rats carbachol produced a concentration-dependent increase in total [3H]-inositol phosphates which was blocked by scopolamine with other agonists behaving as partial agonists. In aged rats the results were more variable. Thus, there were no significant differences between the two age groups comparing dose-response curves and maximal responses, however, the results were more variable. Thus, the previously observed decrease in cortical M1 receptors of aged Fisher 344 rats does not appear to be functionally translated into altered PI turnover.