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*8,138 volunteer abstracts, 17 symposia.
1989 Program Committee

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Yale University School of Medicine

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University of California, San Diego
Medical School
# Chronological List of Sessions

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<td>2. The Neurobiology of Neuropeptide Y (NPY). Chaired by: W.F. Colmers</td>
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<td>3. Functional Organization of the Thalamus. Chaired by: S.M. Sherman</td>
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<td>5. Human behavioral neurobiology: memory</td>
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<td>6. Serotonin</td>
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<td>84. Pharmacology of synaptic transmission</td>
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<td>81. Peptides: biosynthesis, metabolism and biochemical characterization I</td>
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**Poster Sessions—1:00 p.m.**

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<td>91. Peptides: physiological effects I</td>
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<td>99. Oculomotor system I</td>
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<td>100. Biological rhythms and sleep: sleep</td>
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<td>memory and language</td>
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<td>122. Staining and tracing techniques</td>
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**TUESDAY**

**Symposia—8:30 a.m.**

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<td>133. Excitatory amino acids: receptors III</td>
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192. Revolutions and Breakthroughs in Nerve and Muscle.

Sir A. Huxley .................................No abstract

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194. Recent Advances in the Biology of Affective Disorders. *Chairied by:* C.B. Nemeroff .................................479

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No abstract

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Chaired by: P.E. Micevych

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440. Inhibitory Influences on Growth Cones and Cells.  
Chaired by: M.E. Schwab

1106

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442. Biochemical and pharmacological correlates of development III

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443. Alzheimer’s disease II

1112

444. Trauma III

1114

445. Auditory system

1116

446. Invertebrate learning and behavior I

1118

447. Circuity and pattern generation

1120

448. Differentiation, morphogenesis and development: cellular and molecular studies III

1122

449. Mental illness

1124

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1125

451. mRNA regulation V

1127

452. Ingestive behaviors V

1130

453. Monoamines and behavior III

1133

454. Neurochemistry III

1135

455. Invertebrate learning and behavior II

1137

456. Ion channels: cell function

1140

457. Calcium channels: modulation and regulation

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458. Ligand-gated ion channels II

1147

459. GABA and benzodiazepine receptors IV

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**Special Lecture—4:15 p.m.**

492. Microtubules and Cell Morphogenesis. M. Kirschner

No abstract

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**Symposium—8:30 a.m.**

Chaired by: D.N. Krause & M.L. Dubocovich

1255

**Special Lecture—8:30 a.m.**

C. Miller

No abstract

**Special Lecture—10:00 a.m.**

495. Neuronal Organization in the Cerebral Cortex.  
A. Peters

No abstract

**Special Lecture—11:30 a.m.**

496. Signal Processing and Neural Networks in the Oculomotor System. D.A. Robinson

No abstract

**Slide Sessions—8:30 a.m.**

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498. Regeneration II

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**Theme C: Excitable Membranes and Synaptic Transmission**

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

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| 343.           | Brain metabolism and blood flow II                                           | Poster | Wed PM        |
| 17.            | Brain metabolism and blood flow                                               | Slide  | Mon AM        |
| 180.           | Brainstem systems                                                             | Poster | Tue AM        |
| 19.            | Comparative neuroanatomy I                                                    | Poster | Mon AM        |
| 154.           | Comparative neuroanatomy II                                                   | Poster | Tue AM        |
| 165.           | Hippocampus and amygdala I                                                    | Poster | Tue AM        |
| 489.           | Hippocampus and amygdala II                                                   | Poster | Thu PM        |
| 490.           | Hippocampus and amygdala III                                                  | Poster | Thu PM        |
| 431.           | Hypothalamus                                                                   | Poster | Thu AM        |
| 488.           | Limbic system II                                                              | Poster | Thu PM        |

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| 224.           | Alcohol, barbiturates, benzodiazepines IV                                    | Poster | Tue PM        |
| 184.           | Biological rhythms and sleep: invertebrates                                   | Poster | Tue AM        |
| 15.            | Biological rhythms and sleep: neuroregulators                                 | Slide  | Mon AM        |
| 201.           | Biological rhythms and sleep: other I                                        | Slide  | Tue PM        |
| 293.           | Biological rhythms and sleep: other II                                       | Poster | Wed AM        |
| 420.           | Biological rhythms and sleep: other III                                      | Poster | Thu AM        |
| 522.           | Biological rhythms and sleep: other IV                                       | Poster | Fri AM        |
| 100.           | Biological rhythms and sleep: sleep                                           | Poster | Mon PM        |
| 253.           | Drugs of abuse                                                                | Poster | Tue PM        |
| 434.           | Drugs of abuse: CNS pathways                                                 | Poster | Thu AM        |
| 103.           | Drugs of abuse: biogenic amines                                               | Poster | Mon PM        |
| 104.           | Drugs of abuse: cocaine I                                                    | Poster | Mon PM        |
| 322.           | Drugs of abuse: cocaine II                                                   | Slide  | Wed PM        |
| 432.           | Drugs of abuse: dopamine mechanisms                                          | Poster | Thu AM        |
| 469.           | Drugs of abuse: stimulants                                                   | Poster | Thu PM        |
| 155.           | Hormonal control of behavior I                                               | Poster | Tue AM        |
| 304.           | Hormonal control of behavior II                                              | Poster | Wed AM        |
| 435.           | Hormonal control of behavior III                                             | Poster | Thu AM        |
| 130.           | Hormones, Neural Circuits and Communication                                  | Symp.  | Tue AM        |</p>
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Histamine (HA) stimulates spiking when applied to the soma of lobster cells. In voltage-clamped somata, HA activates a Cl- conductance. The Cl-current was unselective for external Co2+ and Cd2+, or internal Ni2+. When coapplied with activators for G-proteins, HA activated a Cl-channel when pulsed to outside-out (-80 mV) but not cell-attached patches (-80 mV). Intracellular GTP-gamma-S did not affect channel gating by HA. These data demonstrate that HA directly gates a Cl-channel with a conductance of 66 pS in the Caribbean spiny lobster and 4.4 pS in the American lobster. The percent of the channel spent in the open state (Pₒ) was a graded function of dose, beginning between 0.1 and 5 µM HA. Some kinetic properties of the channel were sensitive to voltage. The macroscopic current decayed rapidly as a single exponential (τ₀ of about 100 ms) at -70 mV, as a double exponential between -60 and -35 mV (τ₀ of about 100 and 1000 ms), and did not decay below -35 mV. Similarly, the Pₒ of the channel was increased to 10-15 fold when the potential was increased from 0 to 100 mV. The HA-gated channel shares some properties with the GABAA receptor.

Supported by the Grass Foundation, NIMH award K23MH09495 and NSF award BRS8-10261.

EXTRACELLULAR ATP ACTIVATES POTASSIUM CHANNELS IN CHICK SKELETAL MUSCLE. S. A. Thomas and R. I. Hume. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

In developing chick skeletal muscle, micromolar concentrations of extracellular adenosine 5'-triphosphate (ATP) elicit a late potassium conductance in addition to an early excitatory response. The potassium conductance was present with a delay of approximately one second and is greatly reduced at low temperature, suggesting that a second messenger may be involved. In order to examine the mechanism of activation, we voltage-clamped myotubes using the whole patch clamp technique, which allowed us to intracellularly dialyze the cells.

ATP elicited an outward potassium current at the reversal potential for the early excitatory current. The activation of the potassium current was calcium independent, since the magnitude of the current was unaffected by dialyzing cells with 20 mM BAPTA and no calcium. Activation was also independent of G protein alpha subunits, since the current was similar when cells were dialyzed with 20 mM BAPTA and no calcium. Activation was also independent of G protein alpha subunits, since the current was similar when cells were dialyzed for about 100 ms at -70 mV, as a double exponential between -60 and -35 mV (τ₀ of about 100 and 1000 ms), and did not decay below -35 mV. Similarly, the Pₒ of the channel was increased to 10-15 fold when the potential was increased from 0 to 100 mV. The HA-gated channel shares some properties with the GABAA receptor.

Supported by the Grass Foundation, NIMH award K23MH09495 and NSF award BRS8-10261.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

ROLES OF SOMATOSTATIN (SRII) AND GROWTH HORMONE RELLEASING FACTOR (GHRF) IN ETHER STRESS INHIBITION OF GROWTH HORMONE (GH) RELEASE. M.C. Aguilera*, R. Fabián, W.U. and S.M. McCan, Dept. Physiology, Neuropetidios Div., Univ. TX Southwestern Med. Ctr., Dallas, Texas 75235. In order to evaluate the release of somatostatin (SRII) and growth hormone releasing factor (GHRF) into the pituitary gland in response to ether stress, a whole-body perfusion (WBP) technique has been used in freely moving rats. Push-pull cannulae (PPC) were implanted into the anterior pituitary (AP) gland of male rats (200-270 g). After a 7 day recovery period the rats were fitted with lowering jugular catheters. The next day the animals were subjected to PPC of the AP during one hour followed by ether stress (2 min) and another hour of perfusion. The perfusion rate varied from 5 to 10 ml/min and 10 min in the two media were collected and assayed for SRII and GHRF by RIA. Plasma OH levels were sampled every 10 min. At the end of the experiments, the accuracy of PPC tip placements was verified by measuring release of peptide-like molecules as assessed by fluorometric detection. Two conditions SRR and GHRF, and GHRF output pulsed at 20-40 min intervals. SRII and GHRF output in the milligram period beginning with application of ether was increased two-fold (p<0.05). Interestingly, the release of SRII continued for an additional 10 min, whereas GHRF output decreased and was almost undetectable. The release of both GHRF and SRII increased to basal values 30 min after stress. Plasma OH levels were significantly lowered 10 min after stress. Each of the 9 animals showed a restoration of pulsatile OH release to basal levels within 20 to 30 min after stress. The present evidence indicates that somatostatin plays a prominent role in stress-induced inhibition of OH release in the rat by blocking the response to the transient elevation of GH and continuing to suppress OH release for 20 min.

NEUROPEPTIDE Y STIMULATES GH RELEASE FROM SIMIAN HYPOTHALAMUS. K.Y.F. Poon*, A. H. Kaynald and H.G. Spies, (SPON, Y.F. Chen) Oregon Regional Primate Research Ctr., Beaverton, OR 97006. Feeding, cardiovascular, and reproductive functions are modified by neuropeptide Y (NPY) action. In the rat and rabbit, NPY alters the secretion of growth hormone (GH) in vitro and in vivo. We utilized simian (macaque and baboon) hypothalami in vitro in suprenuro studies to examine the release of GH and IGF-I from isolated hypothalami (AH) and the mediobasal hypothalamus (MBH) in response to NPY treatment. Within 3 min after death, blocks of hypothalamic tissue in ice-cold Locke's medium were sectioned between the ventromedial nuclei into left and right halves. The AH/MBH blocks were distinguished rostrally by the anterior commissure and caudally by the mamillary bodies: the MBH and ANH were collected, and the optic chiasma. These 4 fragments (right and left AH, left and MBH) were placed into individual serological chambers submerged in a 4°C water bath and were superfused with Locke's buffer (pH 7.4). Samples (40 µl) were collected at 10-min intervals into tubes containing 40 µl of 1N acetic acid. Hypothalamic fragments received either 6 h of Locke's medium (controls) or 3 h of Locke's medium followed by 3 h of 20 nM of NPY in Locke's medium. GH and IGF-I release from both the AH (n=12) and MBH (n=13) was sustained for the duration of NPY infusion. NPY caused no measurable change in GH release in perfusates of AH (n=10) or MBH (n=9) Exposure to Locke's medium alone for 3 h did not alter GH release from AH (n=6) and MBH (n=7). These results suggest that NPY stimulates GH release via mechanisms that do not involve the release of GH.
A NOVEL HYPOTHALAMIC NEUROPEPTIDE WITH 38 RESIDUES (PACAP38) STIMULATES ADENYLYL CYCLASE ACTIVITY IN PITTUTARY COBALMUTERINE OSTEOCYTES. G. Katsumura, R.R. Dahl*, A. Miyata* and A. Amiraga (SPON: F. R. Dometier), U.S. Japan Biomedical Res. Labs, Tulane Univ. Herbon Center, Belle Chase, LA 70037; and Dept of Medicine & Anaymgy, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

We have recently isolated and characterized a novel hypothalamic neuropeptide with 38 residues that is capable of increasing cAMP levels in rat pituitary cells through cyclic AMP activation. This peptide was named as PACAP38 (Pituitary Adenylate Cyclase Activating Peptide with 38 residues). It was found that PACAP38 had 68% homology with porcine VIP in the C-Termus region 1-28. We examined the effect of synthetic PACAP38 on intracellular accumulation of cyclic AMP in rat pituitary cells, (10^{-5} M) and 10^{-8} M). PACAP38 increased cAMP levels from 2.5 nmoles/200,000 cells to 3.5 nmoles/200,000 cells. PACAP38 was 100-fold more potent than VIP in stimulating cAMP accumulation in rat pituitary cells.

The present study has suggested that PACAP38 may play a role in the regulation of hormone secretion in the pituitary gland. However, the physiological significance of PACAP38 in the hypothalamo-pituitary axis remains to be elucidated.

PARAVENTRICULAR MICROINFUSIONS OF ALL AND AIII IN NORMOTENSIVE RATS INDUCE PRESSOR RESPONSES. Laurie L. Jensen, Joseph W. Harding, and John W. Wetherill. Washington State University, Pullman, WA.; and Depts. of Medicine & Anatomy, Tulane University Sch. of Med., New Orleans, LA 70112.

One source of input to the AMYG is derived from GnRH neurons in the septal area (Janis, Brain, 40: 549-558, 1987). We have recently performed electrophysiological studies to identify AMYG neurons receiving septal input, to determine the influence of estrogen priming on the orthodromic response, and to explore the effect of iontophoretically applied GnRH. Ovariectomized female rats, primed (n=10) or not (n=18) with estrogen (EB, 2ug 44 hrs prior to recording) were anesthetized with urethane (120 mg/kg) and prepared for conventional in vivo extracellular recording. A stimulating electrode was positioned in the medial septal area to orthodromically stimulate AMYG neurons. A multi-barrelled glass pipette was used to record single cell activity in the AMYG and to detect GnRH. Orthodromic responses were analyzed by collecting peristimulus-time histograms. In both primed and unprimed animals, a large percentage of neurons were orthodromically responsive (57 of 103 in primed animals and 46 of 70 in unprimed animals). EB-priming significantly increased the number of orthodromic inhibitory responses. In the small number of GnRH-responsive neurons, no consistent relationship between the GnRH response and the orthodromic response was observed. However, in a few instances, GnRH modulated the orthodromic response; i.e., decreased the amount of orthodromic excitation or inhibition. Although the identity of the neurotransmitter(s) remains to be established, the results demonstrate a substantial, estrogen-sensitive input from the septal area to the amygdala. HD09988 and NIH TG 5T35HL07483-08.

THE ORTHODROMIC RESPONSE OF CORTICO-MEDIAL AMYGDALA (AMYG) NEURONS TO SEPTAL AREA STIMULATION IS MODULATED BY ESTROGEN. C.A. Dudley, Y. Lee*, and R.L. Moss. Dept. of Physiology, UT Southwestern Medical Center, Dallas, Texas 75235.

One source of input to the AMYG is derived from GnRH neurons in the septal area (Jahn, Brain, 40: 549-558, 1987). We have recently performed electrophysiological studies to identify AMYG neurons receiving septal input, to determine the influence of estrogen priming on the orthodromic response, and to explore the effect of iontophoretically applied GnRH. Ovariectomized female rats, primed (n=10) or not (n=18) with estrogen (EB, 2ug 44 hrs prior to recording) were anesthetized with anesthetized with urethane (120 mg/kg) and prepared for conventional in vivo extracellular recording. A stimulating electrode was positioned in the medial septal area to orthodromically stimulate AMYG neurons. A multi-barrelled glass pipette was used to record single cell activity in the AMYG and to detect GnRH. Orthodromic responses were analyzed by collecting peristimulus-time histograms. In both primed and unprimed animals, a large percentage of neurons were orthodromically responsive (57 of 103 in primed animals and 46 of 70 in unprimed animals). EB-priming significantly increased the number of orthodromic inhibitory responses. In the small number of GnRH-responsive neurons, no consistent relationship between the GnRH response and the orthodromic response was observed. However, in a few instances, GnRH modulated the orthodromic response; i.e., decreased the amount of orthodromic excitation or inhibition. Although the identity of the neurotransmitter(s) remains to be established, the results demonstrate a substantial, estrogen-sensitive input from the septal area to the amygdala. HD09988 and NIH TG 5T35HL07483-08.

PARAVENTRICULAR MICROINFUSIONS OF ALL AND AIII IN NORMOTENSIVE RATS INDUCE PRESSOR RESPONSES. Laurie L. Jensen, Joseph W. Harding, and John W. Wetherill. Washington State University, Pullman, WA. 99163. Angiotensin II (AII) immunoreactivity has been reported in cells of the paraventricular nucleus of the hypothalamus (PVH); (Lind & Swanson, 1985), and the microiontophoresis applications of AII and angiotensin III (AIII) has been shown to excite PVH cells with AII inducing significantly larger latencies of activity than AII (Harding & Felix, 1987). This finding was interpreted to suggest that AII may have to undergo conversion to AIII in order to serve as a pressor agonist. In the present experiment anesthetized rats received microinfusions of AII and AIII at doses of 0.1, 10, 50, and 250 pmoles/100 µg in 200 µl of aCSF into the PVH. The figure below indicates dose dependent increases in blood pressure to both AII and AIII, thus extending earlier findings by Brossman et al. (1987) who tested only AII, and supporting the notion that AIII is an important pressor agonist in the PVH. Supported by NIH grant HL32063 & TW01112 and the American Heart Association.

A CENTRAL VASOPRESSINERGIC MECHANISM MEDIATES SALICYLATE BUT NOT ACETAMINOPHEN-INDUCED ANTIPYREXIS. M. Wilkinson and N.P. Kasting. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada.

We have recently demonstrated that the antipyretic action of indomethacin is dependent upon the vasopressin (AVP) V-la-receptor within the ventral septal area (VSA) of the brain. The purpose of this study was to assess the antipyretic effects of sodium salicylate (SALIC) and acetylsalicylic acid (ACETA) alone and in a dose-related blockade within the VSA. Male SD rats were stereotaxically implanted with a chronic cannula and were anesthetized with sodium pentobarbital. They were induced with 30 mg/kg ip. A 2h thermal index was calculated from the time of drug administration for statistical analysis. The V-la-antagonist attenuated SALIC-induced antipyresis in a dose-related manner: saline(SALIC)+SALIC, -0.7±0.2°C hr; 1.0 µg V-la-antagonist(SALIC)+SALIC, -0.5±0.1°C hr (p<0.05); 10.0 µg V-la-antagonist(SALIC)+SALIC, -0.0±0.0°C hr (p>0.01). However, the antipyresis induced by ACETA was unaffected by the V-la-antagonist. Neither dose of the V-la-antagonist or saline within the VSA affected non-fever ileus. These results indicate that, like indomethacin, SALIC but not ACETA activate AVP V-la-receptors within the VSA during drug-induced antipyresis. Supported by the MRC of Canada.

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388.9

A battery of DRG-derived cell lines (ND cells) has been generated by fusing the HAT-sensitive neuroblastoma line NT1/2G2 with neonatal rat DRG cells. After sub-cloning, lines that expressed the rat surface marker Thy 1.1, and the sensory neuron marker globoseries and lactoseries glycolipids were investigated. The sensory neuron-like characteristics exhibited by various lines include expression of δ-opioid receptors, depolarisation in response to capsaicin and susceptibility to latent infection with Herpes Simplex Virus. One line - ND/723 - when differentiated with 1mM dibutyryl CAMP in the presence of 0.5% FCS and NGF, responded to bradykinin with an inward current and an increase in membrane conductance (18/50 cells). The mean latency of the response was 7.5 ±2.1 ± 6.6 sec, the time to peak 47 ±6.8 sec, and the amplitude of the response was 0.2 ±0.05 nA. The reversal potential (n=3) was 11.6 ±6 mV. The characteristics of this response are shared by DRG neurons, and are distinct from the hyperpolarisation with an outward current demonstrated by other neuronal cell lines (e.g. NG108-15). ND/723 cells, like DRG neurons, show elevated levels of cGMP on bradykinin application. Injection of mRNA from rat DRG and ND7 cells confers bradykinin sensitivity on Xenopus oocytes, which depolarise with the characteristics of an IP3-coupled response on bradykinin application. This cell line thus provides a useful model for the analysis of bradykinin-evoked activation of sensory neurons.

388.10
PERIVAGAL APPLICATION OF CAPSAICIN ABOLISHES THE RESPONSE OF VAGAL GASTRIC MECHANORECEPTORS TO CHOLECYSTOKININ. Helene E. Raybould and J.I. Davison. CUR/VA Wadsworth Medical Center, Dept of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073 and Dept of Medical Physiology, University of Calgary, Alberta, T2N 4N1.

Exogenous administration of cholecystokinin-8 (CCK) in anesthetized rats decreases proximal gastric motility and delays gastric emptying partly via a capsaicin-sensitive vagal afferent pathway (Raybould & Tache, Am. J. Physiol. 255, G242, 1988). Recordings were made from single afferent fibers isolated from the cervical vagus of urethane-anesthetized rats (n=8). Gastric motility was monitored manometrically using a catheter placed in the gastric corpus. Four rats were pretreated by perivagal application of capsaicin (1% in 10% Tween 80 in olive oil) under pentobarbital anesthesia 8-12 days prior to experiments. All units (n=20) studied were spontaneously active and increased their discharge in response to gastric distension (2-5 ml). In control rats, all 10 units increased their discharge following intravenous administration of CCK-8 (100 pmol); this was not associated with an increase in intragastric pressure. In capsaicin-pretreated rats, the response to gastric distension was indistinguishable from that obtained in control rats; in contrast only 1 of the 10 units increased their firing frequency in response to CCK-8. Conclusions: CCK stimulates the discharge of vagal gastric mechanoreceptors. The response to CCK, but not gastric distension, is capsaicin-sensitive. These results provide electrophysiological evidence for the capsaicin-sensitive changes in gastric motor function and feeding behaviors following peripheral administration of CCK. HER in receipt of an SKB fellowship. Supported by the MRC Canada.

388.11

Recent results showing the efficient secretion of immunoglobulins by neuronal and glial cell lines (Cattaneo and Neuberg, EMBO J. 6: 2753, 1987) have led to the suggestion that it might be possible to engineer the secretion of specific monoclonal antibodies in the nervous system of an organism in order to perturb or modulate the activity of selected neuronal pathways or cell populations (neuroantibodies). (Cattaneo, Ann. Int. Sup.Santa' 24: 531, 1988). A prerequisite for the neuroantibodies technique is the molecular cloning of the desired monoclonal antibody from the corresponding hybridoma cell line. We now report the molecular cloning of the heavy and light chain of the NC1 monoclonal antibody (Cattaneo and Neuberg, EMBO J. 6: 2753, 1987) that was produced against the neuropeptide substance P (Cuello et al. 1979), with the aim of expressing it in the CNS of a transgenic organism. The derivation and characterization of the NC1 clone will be described, together with the reconstitution of DNA fragments encoding a functional NC1 antibody, in a suitable form for expression in the CNS.

388.12

In the thyroid gland, vasovagal intestinal peptide (VIP) and acetylcholine (ACh) are found in nerve fibers associated with secretory cells and blood vessels. We have, therefore, initiated studies to explore the actions and interactions of ACh and VIP in the regulation of thyroid blood flow (BF) and circulating hormone levels. Previously, we have shown that VIP increases thyroid BF in a dose-related manner. In order to evaluate whether VIP might exert any of its thyroidal effects via muscarinic receptors, we assessed the effects of ACh and VIP in the presence and absence of the muscarinic receptor blocker, atropine. Anesthetized male rats were treated iv with saline or atropine (3 mg/kg) 20 min before iv infusion of vehicle, ACh (3x10^{-5} moles/100gBW), or VIP (10^{-11} moles/100gBW). Organ BFs were measured during this time using radiolabelled {^{38}}Ce microspheres. Mean systemic arterial pressure (MAP) was monitored and used in the calculation of organ vascular conductances (VC=BF/MABP). Atropine pretreatment tended to increase thyroid VC (170.7±37.3 vs. 104.8±19.7 μl/mmHg. min⁻¹·g⁻¹) and the vasodilatory effect of VIP was greater if the rats were pretreated with atropine. When the data were normalized for this stimulatory effect of atropine, the ACh-induced increase in thyroid VC was abolished during muscarinic blockade whereas the vasodilatory effect of VIP was unaffected. These results are consistent with the hypothesis that VIP and ACh exert their effects at the thyroid gland through independent mechanisms and that VIP release may be under prejunctional control via muscarinic receptors. (AM 35037)

Investigations utilizing embryonic chick atria had indicated that adenosine analogs elicit their negative chronotropic effect via an A3 adenosine receptor (ADR). In the present study we have employed the developing chick heart as a model system to investigate the regulation of ADRs. Sustained activation of ADRs was accomplished in vivo (day 9) injections of A3-selective adenosine agonist cyclopentenolodenosine (CPA). Treatment with CPA resulted in a decreased ADR number as measured by the specific binding of the antagonist radioligand β-cyclopentyl-1,3-[3H]dipropylxanthine ([3H]DPCPX). The binding parameters derived from nonlinear regression analysis of [3H]DPCPX saturation isotherms indicated that in vivo treatment with 1 µmol CPA produced a 46±11% reduction in A3 receptors in cardiac membranes. The Kd value for [3H]DPCPX binding to ADRs in CPA treated membranes was not significantly different from the saline treated value (CPA 2.5±0.1 nM; saline 2.1±0.01 nM). The decrease in receptor number was dose dependent suggesting a receptor mediated event. The maximum dose of CPA (10 µmol) resulted in a 77% decrease in the density of adenosine receptors with an ED50 for the CPA-induced down regulation of 2.5 µmol. Administration in vivo of 1 µmol CPA resulted in a decrease in the number of [3H]DPCPX binding sites which was apparent by 4 hours and increased throughout the time span evaluated (24h) when compared to saline treated controls. Co-injection of theophylline was able to attenuate the decrease in AdR number induced by 1 µmol CPA. These data suggest chronic exposure of embryonic day 9 chick hearts to an adenosine analog effect a downregulation of A3 adenosine receptors.

389.2 REGULATION OF CELL SURFACE EXPRESSION AND FUNCTIONAL ACTIVITY OF NICOTINIC ACETYLCHOLINE RECEPTORS ON THE TE671 CLONAL LINE. Anna-M. Jop* and Ronald J. Lukas (SPON. AG. Shetter). Division of Neurobiology, Barrow Neurological Institute, Phoenix AZ 85013.

The TE671 cell line expresses a nicotinic acetylcholine receptor (nAChR) with many similarities to muscle nAChR, yet the regulation of functional activity and expression of nAChR following chronic agonist exposure is clearly different in each system. Effects on cell surface expression (measured as alpha-bungarotoxin binding sites per mg of membrane protein) and functional activity (evaluated using a 6b efflux assay) were assessed using various agents that stimulate or suppress activities of protein kinases A or C or modulate G protein coupling. Dibutyryl cyclic AMP (dbcAMP; 1 mM) treatment induces down-regulation in cell surface nAChR expression within two days of drug exposure whereas the phospholipase C activator, PMA (10 µmol), initially (one day) down-regulates and then increases the number of cell surface nAChR. By contrast, cholera toxin (200 ng/106) induces a rapid (within 5 hours) down-regulation in the number of cell surface nAChR. At these concentrations of PMA, dbcAMP, or cholera toxin, functional nAChR responses (normalized to receptor number) are not changed. These results suggest that second messenger system perturbants have the capacity to regulate levels of nAChR expression without overtly altering nAChR functional activity.


Primary culture of cerebellar granule cells express muacarinic cholinergergic, α1-adrenergic, serotoninergic 5-HT, and histaminergic receptors that couple to phosphoinositol hydrolysis. Exposure of granule cell neurons to a receptor agonist for each of these receptors resulted in time-dependent desensitization of the receptor-mediated phosphoinositide response. The serotonergic receptor was relatively resistant to desensitization and the desensitization appeared to precede the loss of receptor binding sites. Although phorbol esters effectively inhibited the phosphoinositide response mediated by each of these receptors, the degree of evidence speak against the involvement of protein kinase C in the agonist-induced desensitization. Thus, incubation of cells with a protein kinase C inhibitor H7 (25-100 µM) prior to exposure with carbachol, NE, 5-HT or histamine, did not affect agonist-induced desensitization. Depletion of majority of protein kinase C in granule cells by 24 hr incubation with a phorbol ester, PMA or PDB, also did not alter agonist-induced desensitization. Moreover, agonist-induced desensitization was detected even when cells were pretreated with agonists at 4°C; at this temperature, agonist-induced PI breakdown was completely arrested.


It has been suggested that protein kinase C (PKC) is negatively coupled to phosphoinositide hydrolysis because phorbol esters inhibit agonist induced inositol phosphate formation. This hypothesis was investigated by using other agents known to activate PKC via membrane receptors. Formation of [3H]inositol monophosphate ([H-IP] was estimated in embryonic chick sympathetic neurons maintained in culture. Papsychocholine (ACh, 100 µmol) caused 10-fold increase in [H-IP formation. Phorbol 12,13-dibutyrate (PDB) inhibited ACh-induced [H-IP formation in a dose-dependent manner. Furthermore, activation of PKC by serotonin (5-HT, 1 µmol) or ACh (100 µmol) or muscarine (100 µmol) had no inhibitory effects on ACh-induced [H-IP formation. Inhibitory effects of PDB on ACh-induced [H-IP formation persisted even in presence of H7 (1 µmol) or spinosine (100 µmol) which completely blocked PDB activity. Since other activators of PKC failed to mimic and inhibit PKC inhibitors of PKC failed to block the effects of PDB on phosphoinositide hydrolysis, we suggest that phorbol esters may act on sites other than PKC to modulate neuronal metabolism.


Previous studies from our laboratory indicate that the sex steroids, estrogen and progesterone, modulate contractility and other functions regulated by adrenergic β and α receptors, isolated uterine myocytes, and measured β and α adrenoceptor-mediated effects on adenylyl cyclase activity after 16 h in culture. We investigated the regulation of cell surface expression and functional activity of nAChR following chronic agonist exposure is clearly different in each system. Effects on cell surface expression (measured as alpha-bungarotoxin binding sites per mg of membrane protein) and functional activity (evaluated using a 6b efflux assay) were assessed using various agents that stimulate or suppress activities of protein kinases A or C or modulate G protein coupling. Dibutyryl cyclic AMP (dbcAMP; 1 mM) treatment induces down-regulation in cell surface nAChR expression within two days of drug exposure whereas the phospholipase C activator, PMA (10 µmol), initially (one day) down-regulates and then increases the number of cell surface nAChR. By contrast, cholera toxin (200 ng/106) induces a rapid (within 5 hours) down-regulation in the number of cell surface nAChR. At these concentrations of PMA, dbcAMP, or cholera toxin, functional nAChR responses (normalized to receptor number) are not changed. These results suggest that second messenger system perturbants have the capacity to regulate levels of nAChR expression without overtly altering nAChR functional activity.

389.6 MODULATION OF 5-HYDROXYTRYPTAMINE2A, RECEPTOR DENSITY BY GUANINE NUCLEOTIDES. M.A. Harrington and S.J. Peroutka Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

The 5-hydroxytryptamine2A (5-HT2A) is a subtype of the 5-HT receptor which is selectively labeled by [3H]5-hydroxytryptamine. 5-HT, N-methylisopropyladenosine ([H-MIPTA), 4-bromo-5,7-dimethoxyamphetamine ([H-DOB] and [H-DOB] Radioligand binding studies were used to study the effect on affinity (Kd) and Bmax of 5-HT2A. DOI binding in rat cortical membranes pre-exposed to 5-HT was competitively inhibited by 10-4 M ATP, GTP or GTPyS. Co-incubation with either GTP or GTPyS significantly increased the Kd by 57 ± 10%, while co-incubation with GTP and GTPyS significantly decreased the Kd by 37 ± 10% and by 51 ± 10%, respectively. Co-incubation with ATP increased the Kd by 55% and reduced the Bmax of DOI binding, but the changes were not significant.

The change in Bmax observed in the presence of GTP and GTPyS is completely reversible as shown by pre-incubations. Pre-incubation of the membranes with GTP produced no significant change in either the Kd or the Bmax of DOI binding. By contrast, pre-incubation with GTPyS has no effect on the Bmax but significantly increased the Kd by 55 ± 10%. These results suggest that GTPyS enhanced agonist activity which may supprt a different mechanism of regulation in 5-HT2A receptor density and affinity through the actions of G proteins.
389.7 CELLULAR ADAPTATION TO OPTIC EXPOSURE INCREASES AN ADDITIONAL RECEPTOR-BASED PROCESS DISTINCT FROM ELECTRICAL KINDLING IN THE HUMAN POSTMORTEM BRAIN. C. von Euler, P. Mailleux, I. I. Vanderhaeghe, L.F. Agnati and K. Fuxe (SPON: B. Meister). Dept. of Neurochemistry, Stockholm University, Sweden, Lab. of Neurochemistry and Neuropeptide Research, Université Libre de Bruxelles, 808 route de Lenain, B-1070 Brussels, Belgium, and Dept. of Human Physiology, University of Modena, Via Campi 287, 41100 Modena, Italy.

We have previously reported that two weeks following optic nerve transection, NG108-15 cells, following exposure to peptide (DADL) or alkaloid agonist, show increased alteration of agonist binding that can be distinguished from desensitization and receptor downregulation by kinetic and pharmacological criteria. This alteration is selective for peptide agonists; alkaloid agonist and antagonist binding characteristics appear to be largely unchanged. We are most interested in the observation that morphine is capable of reversing this effect completely. By use of a photoaffinity ligand developed recently in our laboratory, we have identified several opiate-binding species and are exploring this receptor-based adaptation further at the molecular level. These findings reveal an additional component of cellular adaptation to optic exposure.

389.8 ENDOGENOUS MODULATORS FOR BRAIN L-GLUTAMATE AND GABA RECEPTORS. J.D. Barcas and C.J. Evans, Pritzker Laboratory of Behavioral Neurochemistry, Stanford University.

We are using NG108-15 cells as a model system to study cellular mechanisms of opiate tolerance and withdrawal. NG108 cells, following exposure to peptide (DADL) or alkaloid (morphine) agonist, show increased alteration of agonist binding that can be distinguished from desensitization and receptor downregulation by kinetic and pharmacological criteria. This alteration is selective for peptide agonist; alkaloid agonist and antagonist binding characteristics appear to be largely unchanged. We are most interested in the observation that morphine is capable of reversing this effect completely. By use of a photoaffinity ligand developed recently in our laboratory, we have identified several opiate-binding species and are exploring this receptor-based adaptation further at the molecular level. These findings reveal an additional component of cellular adaptation to opiate exposure.

389.9 INTRAMEMBRANE REDUCTION OF AFFINITY OF DOPAMINE D-2 RECEPTORS BY CHOLECYSTOKININ-8 AND NEUROTENSIN IN HUMAN POSTMORTEM BRAIN. C. von Euler, P. Mailleux, I. I. Vanderhaeghe, L.F. Agnati and K. Fuxe (SPON: B. Meister). Dept. of Histology and Neurobiology, Karolinska Inst., Box 60400, 10401 Stockholm, Sweden, Lab. of Neurochemistry and Neuropeptide Research, Université Libre de Bruxelles, 808 route de Lenain, B-1070 Brussels, Belgium, and Dept. of Human Physiology, University of Modena, Via Campi 287, 41100 Modena, Italy.

The effects of cholecystokinin-8 (CCK) and neurotensin on the binding of [3H]-n-propylnoraporphine ([3H]-NPA, a D-2 agonist) in cryostat sections and in membrane preparations of postmortem human basal ganglia. CCK decreased the binding of 250 pM [3H]-NPA in the caudate by 50% at 3 nM. Similar, but not statistically significant modulations were seen in the putamen and in the nucleus accumbens. In membranes from the caudate-putamen CCK increased the Kp of [3H]-NPA, by 30% at 10-30 nM without affecting the Bmax value. Neurotensin also increased the Kp without affecting the Bmax of [3H]-NPA. The maximal increase of 45% was obtained at 3 nM of neurotensin. The induction of a reduced affinity of D-2 receptors by CCK and neurotensin are in agreement with results obtained in the rat, and suggest the presence of intramembrane modulation of D-2 receptors by CCK and neurotensin in the living human brain. This receptor-receptor interaction may be of importance for the pathophysiology and treatment of schizophrenia and tardive dyskinesias.


Although chronic intrathecal baclofen has been shown to be an effective treatment for severe spasticity of spinal origin, tolerance does occur in patients over years (Penn, R.D., and Kroin, J.S., J. Neurosurgery, 66:181, 1987). To investigate the possible origin of this decreased sensitivity to baclofen, normal rats were chronically infused with this drug intrathecally to see if GABA receptor binding sites decreased.

Normal rats were implanted with chronic intrathecal catheters and infused for four weeks with either racemic baclofen (0.5 µg/h) or saline vehicle using osmotic minipumps. The dosage chosen was the highest one that did not produce hindlimb muscle weakness. At the end of the infusion period, lumbar spinal cord sections were prepared for receptor autoradiography (Bowery N.G., et al., Neurosci. Abstr., 20:365, 1987). In the substantia gelatinosa, the spiral region where GABA binding sites have the greatest density, the number of sites was reduced by 36% in the baclofen infused animals as compared to the vehicle controls. The results indicate that there is a down-regulation of GABA receptor numbers in the rat spinal cord following chronic high-level exposure to baclofen, and this may be the primary reason for tolerance in patients.
PLASTICITY OF LHRH-IMMUNOREACTIVE FIBERS, IN,
MEDIAL PREOPTIC AREA KINDLING INDUCES SEXUAL BEHAVIOR IN
Fac. of Sci.,Univ. of Tokyo, Tokyo 112.
R.
bulp (AOB) lesion in adult rat. On the other
Lab., Institute Nacional de Neurologia, Mexico, D.F.
days and sexual behavior monitored on even days. The re­
the immunohistochemical technique, if the LHRH-
hand, it has been known that the LHRH-
kindling induces sexual behavior in nonoopulating rats
Sexually Inactive Male Rats. A.E. Haller*, M.C. Manero*,
the animals were stimulated 4 times daily on odd
observed after AMG kindling in noncopulating male rats.

When the kindling technique is used to study the
occurrence and the growth of neuropeptides in the nervous
system, the rapid appearance of immunoreactive fibers
following the kindling stimulation is of particular interest.
To characterize the relationship between kindling
induction and neuropeptide release, studies were
done on the changes in the regional distribution
of LHRH immunoreactive fibers in the olfactory bulb
and the medial preoptic area of the rat. Kindling was
induced in male rats by stimulation of the olfactory
bulb (AOB) or, in some experiments, by stimulation of
the medial preoptic area (MPOA). The rats were perfused
from 2 to 7 days after the kindling stimulation, and the
olfactory bulb and the preoptic area were removed and
immunostained for LHRH. The results show that kindling
induces a change in the pattern of LHRH immunoreactive
fibers in these regions.

The kindling-induced changes in LHRH immunoreactive
fibers in the olfactory bulb appear to be localized to the
peri-inferior olfactory tract, where a marked increase in
the density of LHRH immunoreactive fibers is observed.
This increase is more pronounced when the kindling
stimulation is delivered to the olfactory bulb ipsilateral
to the side of AOB lesion, and it is not observed when
the kindling is delivered to the contralateral side.

In contrast, the kindling-induced changes in LHRH
immunoreactive fibers in the preoptic area are more
diffusely distributed throughout the anterior hypothalamic
area. The distribution of LHRH immunoreactive fibers
in the preoptic area is not significantly different between
the kindled and control sides, except for a slight increase
in the density of fibers in the medial preoptic nucleus.

These results suggest that kindling induces a localized
increase in the density of LHRH immunoreactive fibers
in the olfactory bulb, which may be related to the
sexual behavior induced by kindling. Further studies
are needed to determine the mechanisms underlying
these changes and their role in the induction of sexual
behavior.

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the olfactory bulb and preoptic area of the rat. Brain Res. 483:
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2. Östreich, P., and Haller, A.E. (1987). The role of the
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changes in LHRH immunoreactive fibers in the olfactory bulb
and preoptic area of the rat. Brain Res. 448: 231-239.
DISTRIBUTION OF MYOSIN IN THE CNS IDENTIFIED WITH MONOCLONAL ANTI-MYOSIN ANTIBODIES. M. Morales and E. Figueroa. Department of Pharmacology and Neuroscience, College of Colorado, Boulder, CO 80309.

Previously we have described that polyclonal human anti-myosin antibody cross-reacts with brain myosin in rats and mice. This antibody recognizes myosin in dendritic spines, axon terminals, and in dendrites, an area subserved by the myosin. With this antibody we also observed that myosin is present in all compartments of the neuron, we have developed monoclonal antibodies recognizing brain myosin as an immunogen (in collaboration with the Cancer Center, Health Sciences Center, University of Colorado, Grant P01-NS-00634). The clones were screened for a positive reaction on tissue by immunoelectron microscopy and the specificity of the antibodies was tested on immunoblots (Morales and Figueroa, J. Comp. Neurol. 279:315-322; 1989). With these antisera their distribution in dendrites, axon terminals and dendrites, where it is associated with axon filaments. In addition, we have established that myosin is also associated with microtubules in dendrites and axons. While myosin, in association with axon filaments in dendritic spines, may play an important role in the mechanism of synaptic plasticity, its association with microtubules in dendrites and axons may indicate a transport-providing function.

Supported by NIH grant AI-104129.

KINDLED SEIZURES: AN EVALUATION OF SEIZURE CONTROL PROCEDURES FOR MOLECULAR BIOLOGICAL ANALYSIS. G.Samoriski*, C.D.Applegate, J.L.Burchfiel (SPON:W.L.Gomora). The Children's Hospital, Boston, MA 02115.

One of the major problems in identifying biochemical or molecular biological mechanisms involved in the kindled state has been dissociating changes due to acute ictal events from those responsible for the permanent alterations in seizure susceptibility produced by kindling. Seizure control procedures are necessary to make this dissociation. The ideal procedure should elicit behaviorally identical seizures in naive animals in a single brief trial, and should not result in a positive transfer to the kindled state. In this study we have evaluated 3 seizure induction procedures for electroconvulsive shock (ECS), low frequency train (LFT) stimulation to the frontal cortex, against these criteria. Amygdala implanted rats (6-10/group) were pretreated with one of the seizure induction procedures and following a 1-2 day rest were kindled using standard protocols. ECS elicited seizures were behaviorally distinct from kindled seizures, and did not result in positive transfer. LFP elicited self-sustained stage 5 seizures after 15±1.8m and did not result in positive transfer to amygdala kindling. The relative merits of these procedures will be discussed.

PERSISTENCE OF SOMATIC SPINES TWO WEEKS FOLLOWING RECURRENT LIMBIC SEIZURES IN RATS. M.C. Bundman, R.M. Pico and C.M.Ocal. Departments of Pharmacology and Anxiety and Neurobiology, University of Calif., Irvine CA 92717.

Unilateral, electrolytic lesions of the hippocampal dentate gyrus hilus produce recurrent limbic seizures which begin 2-3 postilesion days. Electron microscopic analysis has demonstrated that during this period of seizure activity there is a dramatic increase in the number of spines on the somata of the dentate gyrus granule cells. At 4 weeks post-HFS, 30±10% (mean ± S.E.M.) of spines had increased in both direction of their number and size. These studies suggest that the increased neural activity experienced during recurrent seizures has long term structural effects in adult CNS neurons. This work was supported by NINCDS NS26748.

Deficits produced by hippocampal lesions are at least partially compensated by housing rats postoperatively in an "enriched" environment. Different results were obtained after entorhinal cortex and fimbria-fornix lesions.

In the present study, we examined the effects of a postoperative environment on the behavior of rats with either entorhinal cortex or dorsal hippocampus lesions on different behavioral tests: spontaneous alternation in a T-maze, learning of an eight-arm radial maze (EARM) and of an Elev-Williams (EWM) maze. Both entorhinal cortex and dorsal hippocampus lesions impaired performance in each of these tasks. Enrichment of the postoperative environment failed to facilitate behavioral recovery of any of the entorhinal cortex lesion-induced effects. Similarly, postoperative enrichment failed to attenuate the deleterious effects of dorsal hippocampus lesions on spontaneous alternation. However, in the same rats, the "enriched" housing attenuated the deficits observed in the EARM maze just at the P < 0.05 level (P = 0.44) and in the EWM maze at the P < 0.01 level (P = 0.24).

Thus, postoperative effects appear to be both TASK and LESION-LOCUS SPECIFIC.


Ablation of the entorhinal cortex (EC) of the rat induces a complex sequence of dendritic reorganization and axonal sprouting in the denervated dentate gyrus (DG). The signal that triggers these events is unknown. One candidate, however, is the reduction of granule cell firing which follows the EC lesion (Reeves and Stewart Exp. Neurol. 102:37-49, 1988). As a first step in confirming these observations, we determined whether the EC lesion was sufficient to silence the dentate EC input in vivo. Under urethane anesthesia, we isolated putative granule cells with a tungsten electrode. After defining baseline activity, we injected 0.2 to 0.4 of 2×10^(-7) M TTX in 0.5% NaCl into each of 14 stereotactic sites along two parallel tracks in the EC (Gyrus). In all cases, the TTX injection reduced granule cell firing in the injection site to baseline levels for 6-8 hours. We found that TTX injection reduced granule cell activity to the same extent as did EC ablation. There was no recovery of evoked responses or cell firing in the hours after the injection. These observations did not alter granule cell activity.

We conclude that the previously described rate changes reflect, in the short term at least, lost presynaptic drive rather than an indirect metabolic effect of degeneration or an effect dependent upon early degenerative events in presynaptic terminals. Biochemical and morphological studies will determine if silencing the EC reproduces the effects of degeneration. Supported by NSF BNS-8916029 (GS). RT received NIH postdoctoral training grant 5T32NS07199.


Thyroid hormone dysfunction at an early stage of development produces marked neurochemical and morphological changes in the hippocampus. In order to better understand the functional significance of these effects, we examined LTP induction in the dentate gyrus (DG) of thyroid hormone-treated and control rats after silencing the dentate EC input. Thyroid hormone (3, 5, 3'-triiodo-L-thyronine (T3)) was administered to male rats on postnatal days 1, 2 and 4 (0.5 μg/g of body weight) at approximately 2 months of age. The two groups of animals were tested for the induction of LTP in the DG gyrus at the P < 0.05 level (F 1/21 = 4.4) and in the EWM maze at the P < 0.01 level (F 1/21 = 24.47). Therefore, the present results suggest that brief T3 treatment of the PP produced a slight increase in the slope of the LTP induction in the dentate gyrus (DG) of thyroid hormone-treated and control rats after silencing the dentate EC input. However, the population spike of the control group increased significantly (44.7% +/- 3.5 SEM) following the perforant path (PP) and recording in the DG gyrus for 3 months. In both the control and T3 groups, tetanization of the PP induced a slight increase in the slope of the EPSP (5.0% +/- 2.0 SEM and 5.2% +/- 3.43 SEM, respectively). However, while the population spike of the control group increased significantly (44.7% +/- 3.5 SEM) following the perforant path (PP) and recording in the DG gyrus for 3 months, the population spike of the T3 treated rats decreased significantly (44.7% +/- 23.0 SEM). The present results suggest that brief T3 treatment at a critical age may have deleterious long-term changes in hippocampal physiology and function. Supported in part by NSF grant BNS 8706653 to J. Winson & grant M41256 to BSN.


Unilateral EC lesions impair performance on a learned alternation task from which the rats recover postlesion. Since the time course of this recovery parallels the time course of sprouting, these two phenomena are thought to be related (Kelche and Stewart, J. Neurosci., in press). The objective of the present investigation was to determine whether there are limits within which sprouting may affectively contribute to recovery. We examined the effect of unilateral EC lesions on a spatial alternation task (Y maze) with intertrial intervals of differing lengths (0, 40, 70, 100 sec). Whereas the performance of the EC group was spared, the other groups showed a deficit from which they all required 10-12 days to recover. This finding indicates that the effects of EC lesions on spatial alternation depend upon task difficulty as determined by the length of the intertrial interval. Since the time course of recovery was comparable for the 40, 70, and 100 sec groups, such recovery evidently is independent of task difficulty. The known parallel between the time course of recovery and sprouting after EC lesions therefore implies that the recovery depends upon the sprouting instead. Supported by NIH grant NS29498 (J.J.R.).

LOW-FREQUENCY DEPRESSION MODULES LONG-TERM POTENTIATION OF THE PERFORANT PATH IN DISINHIBITED IN VITRO DENTATE GYRUS. P. C. Rinaldi and E. M. Hookano*. Neurophysiology Lab, Div. of Neurological Surgery, College of Medicine, Univ. of California at Irvine, Irvine, CA 92717.

Low-frequency depression (LFD) was studied in the disinhibited in vitro dentate gyrus (rat) to determine its role in modulation of long-term potentiation (LTP) and thus its importance in computational and theoretical considerations of learning and memory. An electrode placed in the trajectory of the perforant path inputs to the granule cells delivered high-frequency stimulation (400 Hz) to induce LTP and low-frequency stimulation (0.5 Hz) to the dentritic field potential was recorded to assess synaptic activity. Following the induction of stable LTP in 11 slices (F increase 52%), low-frequency stimulation was delivered to the same inputs for 3 to 10 minutes. During the course of this stimulation synaptic depression averaged 29%. The post-LTP/LFD response amplitude level was significantly reduced when LFD followed LTP. LFD appears to be capable of modifying or reversing LTP in the dentate. It may play a role in modulation of information in neural networks, particularly in extinction or forgetting. (Supported by NIH NS22980-01A1 to PCR.)

HIGH AFFINITY CHOLINE UPTAKE AT THE RAT HIPPOCAMPAL SYNAPTOSOMES IN RESPONSE TO ACUTE EXERCISE BOUTS OF TREADMILL RUNNING. D. E. Fordyce and R. H. F. Farrar. Dept. of Kinesiology and Institute for Neurosciences, University of Texas at Austin, TX 78712.

High affinity choline uptake (HACU) is the rate limiting step in acetylcholine synthesis. A variety of interventions have been demonstrated to affect acetylcholine turnover and synthesis. Previously we have observed that endurance exercise for 6 months, resulted in a 30% decline in HACU when compared to sedentary age-matched controls. All rats had been sedentary for 24 hrs before being killed. It was of interest to determine whether this depression in HACU was due to the last bout of exercise or whether it represents a training adaptation. Therefore, rats were run for 3-4 months on a treadmill (5 min/day, 3 days/week). The treadmill was divided into four groups: two groups ran 25-30 min at a speed of 20 m/min, group R was killed immediately at the end of a 1-h run; group C was killed after 24 hrs before decapitation, and the third group was familiarized with the treadmill, but had not been exposed to the treadmill for 48-72 hours prior to decapitation, the fourth group was a naive control. The HACU was determined on hippocampal synaptosomes incubated with 0.75 μM [3H]-choline, with and without sodium. There was no difference between the naive controls and the familiarized controls and these values are represented as control values (C). Both the R and R24 synaptosomes demonstrated a 50% increase in HACU compared to the control values. These data indicate that acute bouts of treadmill running elevated acetylcholine synthesis in the hippocampus, but that chronic endurance running produces a compensatory reduction in this synthesis.
390.19

RESPONSES TO VARYING INTENSITIES OF VAGINAL DISTENSION IN THE AWARE RAT. K. J. Berkley and E. Wood*. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

It is known that sensory fibers in the pelvic nerve of anesthetized rats respond to gentle distension of the vaginal canal and that the responses increase as distension is increased into the noxious range. In order to examine the relation between this neural response and sensation, similar vaginal distension stimuli were delivered to rats that had previously been trained to perform operant escape response to terminate a noxious somatic stimulus (tail pinch). At gentle levels of distension, rats oriented towards the stimulus, but failed to make escape responses. As the levels were gradually increased into the noxious range, however, the probability of the rats making an escape response gradually increased to 100 percent. This correspondence between electrophysiological and behavioral responses is consistent with the hypothesis that both pain and non-painful sensations arising from mechanical stimulation of the vaginal canal are subserved at least in part by activity in different fibers of the pelvic nerve.

Supported by NIH grant NS 11892.

LIMBIC SYSTEM

391.1


While gonadal steroids are known to influence hippocampal neuronal structure during development and in response to injury, steroid-mediated morphologic plasticity in the intact adult hippocampus has not previously been demonstrated. We have used the single section Golgi impregnation method to show that removal of circulating gonadal steroids by ovariectomy of adult female rats results in a profound decrease in dendritic spine density in CA1 pyramidal cells of the hippocampus. Spine density in CA3 pyramidal cells and granule cells of the dentate gyrus is unaffected. Estradiol replacement reverses the observed decrease in dendritic spine density; progesterone augments the effect of estradiol within a short time period. These results demonstrate that adult CA1 hippocampal pyramidal cells are structurally plastic and suggest that dendritic morphology may undergo constant fluctuation during the estrous cycle.

391.2

EFFECTS OF NORADRENERGIC AGENTS ON PYRAMIDAL NEURONS IN IMMATURE RAT HIPPOCAMPU. A.M. Moudy and P.A. Schwartzkroin. Departments of Physiology & Biophysics, and Neurological Surgery, University of Washington, Seattle, WA 98195.

In preliminary experiments, we have examined the effects of a noradrenergic agonist on individual hippocampal pyramidal neurons from immature rats. Transverse hippocampal slices (400 µm) were obtained from 7 day old Sprague Dawley rats and maintained in an in vitro interface recording chamber at 35°C. Intracellular recordings were made from pyramidal cells in the CA1c and CA1 regions of the hippocampal formation. Isoproterenol, a B-receptor agonist, was pressure-applied from the tip of a glass microelectrode positioned near the soma, as closely as possible to the intracellular recording electrode. Pressure pulse applications (30 psi) of 0.1 mM isoproterenol were given for 50-300 ms. Most cells recorded from both CA1 and CA3c were sensitive to drug application. Voltage changes induced by isoproterenol were variable, however; while some cells showed a slight hyperpolarization, others were depolarized. An input resistance increase of 10-20 Mohms was seen during these responses in a majority of cells. Trains of action potentials triggered by 200 ms depolarizing current pulses showed accommodation in all cells under control conditions; isoproterenol reduced the degree of accommodation, and also reduced the after-hyperpolarization following these trains. These changes were, for the most part, qualitatively similar to noradrenergic effects in adult tissue.

Supported by NIH grants NS 15137 and NS 07097.

391.3

THE DEVELOPMENT OF THE SYNAPTIC PAIRED-PULSE PROFILE IN AREA CA1 OF THE RAT HIPPOCAMPAL SLICE PREPARATION. II. STRATUM MOLECUARE. E.G. D'Arcy and J.J. Trest Neurobiology Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We study the development of the trisynaptic circuit by examining the development of excitatory and inhibitory synaptic transmission in the dentate gyrus and hippocampus proper. While studying the development of paired-pulse facilitation in stratum radiatum of CA1, we noted that afferents in the distal apical dendritic field showed an unusual paired-pulse profile (PPP; 10-5000 ms IPI) during development. We observed a triphasic pattern with paired-pulse depression at short interpulse intervals (IPI), facilitation at intermediate IPIs and a depression at long-latency IPIs. This pattern mirrors the synaptic PPP produced by the med. perforant path input to DG rather than the profile in CA1c. We investigated this effect more closely. The rat hippocampal slice prep and a homosynaptic paired-pulse paradigm in vitro interface recording chamber were used to examine the development of the synaptic PPP in stratum moleculare of area CA1. Population EPSPs were evoked by str. moleculare stimulation at 40-60% max. Our initial results are based on two animals at each age. The paired-pulse effects ((Test/Cond)x100) developed as follows:

<table>
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<tr>
<th>Age (days)</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
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<tr>
<td>P4</td>
<td>64%</td>
<td>77%</td>
<td>85%</td>
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391.4

PHARMACOLOGY OF RETINOTECTAL TRANSMISSION IN RANA PIPIENS TECTAL SLICES. P. W. Hickmott* and M. Constantin-Paun (SPON: J. Paan) Dept. of Biology, Yale Univ., New Haven, CT 06511.

We have developed a tectal slice preparation to examine the pharmacology of retinotectal transmission in Rana pipiens tadpoles. We use 500um thick slices of the diencephalon and tectum. By electrically stimulating the optic tract, which runs through the diencephalon, we can record tectal evoked potentials (TEP) similar to those seen in the intact animal. Such TEPs consist primarily of a fast biphasic component, followed by slower positive and negative components. We have identified the fast biphasic component as postsynaptic on several criteria: 1) it is relatively insensitive to zero Ca++ and Mg++, balancing salting; and also to high concentrations of the calcium channel blockers CaO4 or CdCl2. 2) it is not abolished by high-frequency stimulation and recovers immediately after a stimulus. 3) it does not exhibit paired pulse facilitation. 4) it exhibits a slight hyperpolarization when blocked when transmission is blocked by Cd++. We conclude that the later components of the TEP are postsynaptic.

Furthermore, low concentrations of NMDA reversibly block the postsynaptic components, while low concentrations of APV reversibly enhance them. Concurrent with the postsynaptic block due to NMDA, there is a marked increase in spontaneous activity in the tectum, and a substantial increase in the evoked postsynaptic components. Since the increase in the presynaptic component is not blocked when transmission is blocked by Cd++, it is specific to presynaptic elements. We hypothesize that NMDA receptors are highly concentrated on inhibitory cells, which, when driven by exogenous NMDA, inhibit the postsynaptic TEP. Preliminary evidence further suggests that GABA may be involved in the actions of NMDA on the TEP.

Supported by NIH grant EY00393.
SIGMA RECEPTOR RECOVERY FOLLOWING MODIFICATION BY EEDQ:
EVIDENCE FOR DIFFERENT AGONIST AND ANTIGEN RECOGNITION SITES ON OR RECEPTOR SUBTYPES. E. Campagna, C. L. Keenan. Department of Pharmacology, Loyola University, Maywood, IL 60153

Sigma receptor recovery following irreversible inactivation by β-ethoxy-carbonyl-2-ethoxy-1,2-
dihydroquinoline (EEDQ) was studied. Male SD rats were treated with either vehicle or EEDQ (10 or 20 mg/kg, i.p.) and sacrificed at 6 hours or 4 days post-treatment. Other groups of animals were pretreated with 3 mg/kg of either the agonist DFG (1,3-di-O-tolyl-guanidine) or the antagonist haloperidol (HAL) prior to 10 mg/kg EEDQ to assess recognition of the agonist or antagonist, respectively. The binding of [3H]-DTG and [3H]-HETG to brain membranes were assessed by using the voltage response to current steps. Synaptic properties were assessed by depolarizing current steps and measuring the time that the membrane potential for 500 ms. The ability to obtain structure-function relationships in this heterogeneous cell population should prove useful in understanding the circuitry and the motoneurons of the amygdala. (Supported by the Air Force Office of Scientific Research and the Office of Naval Research).

Preliminary results indicate opioid activity throughout the myocardium of the dog. Opioids were extracted from the organ using a radioimmunoassay (RIA) method using canine heart tissue. Preliminary studies have demonstrated that the opioid activity is present in the canine heart, and further studies are planned to investigate the role of opioids in cardiac function.

OPIOID PEPTIDE SCREENING OF DOG HEART EXTRACTS. B.A. Barron, J.F. Gauz*, J.C. Caffe*, Department of Physiology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.

Opioids in dog heart extracts are characterized by opioid receptor binding assays using canine heart tissue. The study provides evidence for the presence of opioid peptides in canine heart extracts. Future studies will investigate the role of these opioids in cardiac function.

EFFECTS OF OPIOID RECEPTOR BLOCKADE ON HIPPOCAMPUSS DEVELOPMENT IN ADOLESCENT RATS. J. Reyes*, D. Fish*, and M.C. Diamond. Department of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Studies have indicated that the CNS is increased in size and cellular content when opioid receptors are blocked by Naltrexone. Our studies examine the relationship between opioid receptor blockade, size (thickness) and laterality in the hippocampus of 41-day-old rats. Male and female Long-Evans rats were injected subcutaneously with either saline, 1, 3, or 10 mg/kg of Naltrexone, an opioid antagonist, in the first 41 days of postnatal life. On day 41 rats were sacrificed and morphometric analysis of the hippocampus was made on transverse, frozen 40-µm sections stained with a modified standard procedure. Results indicated increases in male hippocampal thickness at each dosage (p<0.05) when compared to control (saline) rats. Furthermore, significant lateralization differences were found in the 3 mg/kg group, with the left hippocampus being larger than the right (p<0.05). These results are opposite to those seen in male rats treated for the first 21 days postnatally. Female rats showed no significant differences between dosage groups in hippocampal size or laterality. However, as the dosage of Naltrexone increased, the left hippocampus progressed from smaller than controls at the 1 mg/kg dosage to larger than controls at 10 mg/kg. These results are similar to what is seen in females exposed for 21 days postnatally to Naltrexone.

BIOCHEMICAL CHARACTERIZATION AND BRAIN DISTRIBUTION OF H-NALOXONE BINDING SITES IN A URODELE AMPHIBIAN. E. Mota and F.L. Houck.

PREP. AT AL, Department of Anatomy, Univ. Alaska Fairbanks, Fairbanks, AK 99775 and Dept Zool Oregon St Univ. Corvallis, OR 97331.

Suspensions of partially purified intermediate cells prepared from newt (Taricha granulosa) brains were incubated with H-naloxone and bromocriptine. H-naloxone binding was found to be specific and saturable (KD: 1.3+0.7 nM, Bmax 155+29 fmol/mg protein). Opioid receptors require monovalent cations for GTP-regulation. Our results are similar to those obtained in rat brain membranes. Therefore, present many similarities.
KAPPA OPIOID RECEPTOR-MEDIATED PHOSPHOSPHODIESTER TURNOVER IN RAT BRAIN. S. Periyasamy and W. Horst (SPONSOR: H. Rosenberg), Department of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH 43606.

Both biochemical and pharmacological evidence support the existence of at least three subtypes of opioid receptors, μ, δ, and κ, in the CNS. Opioid receptors, including κ-receptors, are associated with the inhibition of adenyl cyclase, stimulation of low-K⁺ GTP hydrolysis and more recently with the regulation of K⁺ and Ca²⁺ channels. However, opioid receptors have not been previously associated with the phosphodiesterase turnover response. We have investigated the effects of various subtypes of opioid agonists and antagonists on PI turnover response in the rat brain. U-50,488H, a selective κ-receptor, enhanced the hydrolysis of inositol phospholipids, as reflected by increased [3H]-inositol phosphate (IP) formation in rat hippocampus, precluded with [3H]-IP and treated with L-732. A selective agonist U-50,488H kainocyclohexyl and DAlA2-dynorphin A (1-13) amide produced a concentration-dependent increase in the accumulation of IPs in hippocampal slices. The increase in IP formation elicited by U-50,488H was partially antagonized by naloxone and more completely antagonized by the κ-selective antagonist non-benzylmorphine and MR 2266. The formation of IPs induced by U-50,488H varies across different regions of the brain, being higher in hippocampus and amygdala and lowest in striatum and pontomedullary. The results indicate that brain κ- but not μ- or δ-receptors are coupled to the PI turnover response in the brain. It is suggested that the effects of dynorphin in brain are mediated by receptors linked to the PI turnover response.Supported by DA04688.


Opioids regulate neuroblastoma growth via receptor mediated mechanisms. A current experimental model is the murine neuroblastoma line N18TG2, but the stability of its expression of opioid receptors in vivo has not been reported. N18TG2 cells were injected into male nude mice and the tumors passed serially. At each generation, tumors were excised at necropsy and analyzed for opioid receptor binding parameters using [3H]FOXY (New England Nuclear, SA=40.1 Ci/mmol) as previously described. The binding parameters using [3H]FOXY revealed no morphological differences between the original and recultured cells. Repassaging the recultured cells in vivo led again to a rapid decline in opioid receptor expression. The Kd for DADLE (2.5 nM) remained unchanged. Opioid receptor expression is thus a non-constitutive property of N18TG2. This alteration of receptor expression represents an important variable and may prove useful in delineating the mechanism of opioid modulation of tumor growth.

PRETREATMENT OF RATS WITH THE IRREVERSIBLE µ-RECEPTOR ANTAGONIST, β-FNA, FAILS TO PREVENT NALTREXONE-INDUCED DEPRESSION OF µ-OPIOID RECEPTORS. A. Artroffman, J. O. Lona, S. Bykoff, K. C. Rice and J. W. Rees. Unit on Nicotine and Drug Abuse, NIMH, Bethesda, MD 20892.2

β-FNA is a μ receptor antagonist and naltrexone is a μ receptor antagonist. The effect of β-FNA on μ opioid receptor binding was studied in vitro and in vivo. In vitro, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. In vivo, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. These results suggest that β-FNA is a μ receptor antagonist and naltrexone is a μ receptor antagonist. The effect of β-FNA on μ opioid receptor binding was studied in vitro and in vivo. In vitro, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. In vivo, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. These results suggest that β-FNA is a μ receptor antagonist and naltrexone is a μ receptor antagonist. The effect of β-FNA on μ opioid receptor binding was studied in vitro and in vivo. In vitro, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. In vivo, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. These results suggest that β-FNA is a μ receptor antagonist and naltrexone is a μ receptor antagonist. The effect of β-FNA on μ opioid receptor binding was studied in vitro and in vivo. In vitro, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. In vivo, a significant reduction in μ binding sites was observed in brain tissue treated with β-FNA. These results suggest that β-FNA is a μ receptor antagonist and naltrexone is a μ receptor antagonist.

Met-enkephalin (Enk) was used as an antibody (N82) with a crossreactivity of 31%, 25, 11, and 5% to Lys6, Arg6, Arg6-Phenyl, and Arg6-Leu7 peptide terminus extensions of Enk, respectively. Pigs (n=6) were dissected immediately postmortem into 111 brain, cor pulmonale and ganglia sections, and extracted in 0.1 M HCl. Total encrypted Enk was also evaluated in brain regions after trypsin/carboxypeptidase B treatment extracts, and ratios of total Enk/Enk calculated.

392.17 STRUCTURE-ACTIVITY RELATIONSHIPS OF DFG AND ITS CONSERVING AT THE HALOEPIDOXIC-SENSITIVE SIGMA RECEPTOR. B. Testa*, M. Schubert, E. Weiler, V. Willoughby, Oregon Health Sciences University, Portland, OR 97201 and Department of Pharmacology, Oregon Regional Primate Research Center, Beaverton, OR 97005 (Supported in part by NIDA #DA02265 to H.A. and a MRC Fellowship to D.B.).


Kappa opioid receptors have been characterized by their binding and pharmacological properties, but the question of whether second messenger systems are coupled to kappa receptors is not yet settled. Both mu and delta opioid receptors inhibit adenylate cyclase in brain membranes. Guinea pig cerebellum, which contains kappa receptors but lacks mu or delta mechanisms, has been a useful system for studying adenylate cyclase inhibition by kappa opioids. In these experiments we studied the effects of dynorphins on adenylate cyclase activity in membranes from guinea pig cerebellum. Membranes were prepared from guinea pig cerebellum and preincubated at pH 4.5 to increase inhibitory activity. Adenylate cyclase was reactivated using forskolin and met-enkephalin as controls.


The expression and regulation of proenkephalin, proopiomelanocortin, and prodynorphin has been investigated in primary cultures of purified glial cells from the neonatal rat cerebral cortex. Northern blot analysis revealed that proenkephalin gene expression is highest in astrocytes, and that proenkephalin expression is not detected in bipotential glial precursor cells or neurons. Proenkephalin expression in primary cultures of purified glial cells from neonatal rat cerebral cortex. Northern blot analysis revealed that proenkephalin gene expression is highest in astrocytes, and that no proenkephalin expression was detected in bipotential glial precursor cells or neurons.

392.20 AGE-DEPENDENT SUBCELLULAR DISTRIBUTION OF OPIOID RECEPTORS AND G-PROTEINS IN RAT BRAIN. C.J. Coscia, S.J. Yeung, M. Belcheva, and W.T. Ben. Dept. of Biochem., St. Louis Univ. Res. Ctr., St. Louis, MO 63104. Evidence for ontogenic differences in binding and structural properties of rat brain opioid receptors has been reported previously. In the present study we investigated whether age-related differences in the localization of opioid receptors exist. No opioid receptor expression was detected by immunohistochemical or biochemical methods in glial cells from the neonatal rat cerebral cortex. Northern blot analysis revealed that all three opioid gene products were expressed in astrocytes, and that proenkephalin gene expression was highest in astrocytes. No proenkephalin expression was detected in bipotential glial precursor cells or oligodendrocyte progenitor cells. Treatment of the astrocytes with 10^6 M serotonin for 24 hours upregulated the expression of the opioid receptor gene.

THURSDAY AM

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
CHRONIC NALTREXONE INCREASES EXPRESSION OF PREPROENKEPHalin AND PREPROTACHYKININ mRNA IN DISCRETE BRAIN REGIONS. K.S. Zakin, J.A. Keplinger and A. Yapici (SPON: A.B. Johnson). Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Long-term blockade of opioid receptors by the antagonist naltrexone increases met-enkephalin in the striatum and nucleus accumbens (Templ et al., 1984). To determine whether these changes reflect increased peptide levels, we examined preproenkephalin (PPE) mRNA content in brain regions of control (placebo-treated) and naltrexone-treated animals by Northern analysis. Naltrexone treatment (8 days) led to a 1.5-fold increase in striatal PPE mRNA. No statistically significant change was observed in striatal cyclinoid (BI/S) or azim mRNA. Small increases in PPE mRNA occurred in the hippocampus (+40%) and hypothalamus (+19%). Increases in mRNA occurred 24 hrs following onset of antagonist, increases in opioid receptors were also maximal 3-4 days after onset of antagonist. To determine whether substance P synthesis is altered by naltrexone, preprotachykinin (PPT) mRNA content was examined. Naltrexone (8 days) increased striatal PPT mRNA by 5-fold. Striatal substance P was increased 3-fold. These findings suggest that chronic blockade of opioid receptors leads to increased synthesis of both enkephalin and substance P in the striatum, and that the changes are relatively specific. The finding of opioid antagonist-induced increases in PPT mRNA and substance P support the concept of a role for enkephalin in the regulation of substance P gene expression. [Supported by NIH grants DA04439 (R.S.Z.), DA05440 (A.T.) and NS20718 (J.A.K.)].

Choline acetyltransferase (ChAT) is the biosynthetic enzyme for the neurotransmitter acetylcholine. It has been observed that nerve growth factor (NGF) increases ChAT activity 3-5 fold in cultured fetal septal neurons (Hefti et al., Neuroscience 14: 55, 1985). In order to study this phenomenon further, we have identified a human genomic clone corresponding to ChAT using oligodeoxynucleotides directed against porcine and human ChAT (Hersh et al., this volume). A 2.8 kb Bam H1 fragment of this clone coding for the amino terminal region of the ChAT protein cross hybridizes with a 4000 nt rat mRNA on Northern blots, in agreement with Berrard et al. (PNAS 84: 9280, 1987). This RNA species was detected in poly A (+) RNA isolated from rat basal forebrain, cortex, and septum, but not from hippocampus. We currently are examining the effect of NGF on ChAT RNA levels in cultures of fetal rat septal neurons.

393.4 IN SITU QUANTITATION OF CEREBELLAR GAD mRNAs DURING RAT BRAIN DEVELOPMENT AND IN NERVOUS MICE. K. Grasham, M. Willcutt, R.W. Rosenberg and R.R. Morrison-Bogorad. Departments of Neurology and Biochemistry, U.T.-Southwestern Medical Center, Dallas, Texas, 75235 (Spon: J.L. Steinberg).

We have quantitated GAD mRNA levels in the different GABAergic cell types of the cerebellum and compared these to the levels of intracellular polypeptide. In normal rat and mouse, all Purkinje cells contain GAD mRNAs, although the amount in individual Purkinje cells fluctuates. The relative amount of GAD mRNA in different cell types does not differ more than 3 fold. In adreno-cerebellar mice, (90% of Purkinje cells have degenerated. The remaining Purkinje cells have approximately the same levels of GAD mRNA as those in normal mice. GAD mRNA levels are also still high in the other GABAergic cell types. In developing rat cere­bellum, GAD mRNA levels in the prematurely-formed Purkinje and Golgi cells is already high by P14. No GAD mRNAs are seen in the ECL and they are infrequent in the proximal parts of the molecular layer. GAD mRNA in stellate and basket cells increases as the cells migrate towards the IGL. These results show that GAD mRNA levels in Purkinje cells do not fluctuate greatly, either under normal physiological conditions or when Purkinje cell number is greatly depleted. Developmentally, GAD mRNAs are rapidly induced in Purkinje and Golgi cells and are induced in stellate and basket cells as they migrate through the molecular layer. Supported by NIH 18486 (MB-B) and Zale Foundation (BRM).

393.5 GABA RECEPTOR MODULATION OF TYROSINE HYDROXILASE GENE EXPRESSION IN THE ADRENAL GLAND. C. Hale, M. Wessels-Reiter, M.A. Moore, R. Zoeller, and J. Flood. Geriatric Research, Education, and Clinical Center, St. Louis VA Medical Center and St. Louis University of Medicine, St. Louis, MO 63125

Several studies demonstrated the presence of GABA receptors on chromaffin cells of the adrenal medulla. Furthermore, other studies suggest that GABA receptors in the adrenal gland are important regulators of catecholamine biosynthesis. We have shown by in situ hybridization, that the GABA receptor mRNA is expressed in chromaffin cells. Furthermore, bicuculline has been shown to increase the release of catecholamines elicited by splanchnic nerve stimulation. These studies suggest that GABAergic pathways which increase catecholamine release from the adrenal often induce TH gene expression. In order to test this possibility, we have examined the role of GABA receptors in plasticity of TH gene expression.

We treated rats with a single injection of reserpine (7.5 mg/Kg/s.c.) or the GABA antagonist bicuculline (10mg/Kg/s.c) twice a day for three days, or a combination of a single reserpine treatment and treatment with bicuculline three times. At the end of three days the animals were sacrificed and the adrenal and brains were rapidly removed for quantitation of TH enzyme and TH mRNA by Radioimmunoassay and Northern blot. These data suggest the possibility of GABA mediated modulation of TH gene expression.


To determine whether the reported differences between adrenalin TH activities of SHR and WKY rats are associated with changes in TH gene expression, we have studied levels of TH mRNA in the adrenals of these two strains of rats (nine weeks old). The levels of adrenalin TH mRNA were determined by Northern blot analysis. The total mRNA was fractionated by gel electrophoresis, transferred to Gene Screen membranes, and baked at 80°C. The immobilized mRNA was hybridized with random primed TH cDNA (Pat 1) of 742 bp. The hybrids were detected by autoradiography and the density of the bands were determined. The levels of TH mRNA were found to be 30-50% higher in adrenals of WKY than that of SHR rats. These results support the previously reported findings that TH activity is higher in adrenals of WKY than in SHR rats (H. Groeber et al., Nature 258:267-268, 1975). Supported by NIMH 02717 and NINCDS 06801.


The 5' coding region of human DBH was cloned using the polymerase chain reaction (PCR) and sequenced by dideoxysequencing. The predicted amino acid sequence was detected on 3% agarose gels and gel purified fragments were sequenced to verify their identity. Transcripts of these DBH templates were synthesized using T7 RNA polymerase and using a reticulocyte lysate system. This new PCR/translation/translation system is being utilized to study the potential translational regulation of the nascent amino terminus of DBH by unedited nascent terminus.

393.8 MODULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION IN SUBREGIONS OF THE LOCUS CERULEUS. B. Strong, C. Hale, M. Moore, M. Wessels-Reiter, and R.T. Zeeler (SPQR: J. Flood) Geriatric Research, Education, and Clinical Center, St. Louis VA Medical Center and St. Louis University School of Medicine, St. Louis, MO 63125 and University of Missouri Medical School, Columbia Missouri.

The role of neurotransmitters and receptors in the reserpinized elicited increases in TH mRNA and TH enzyme in the locus ceruleus is unknown. Furthermore, the neurochemical nature of many of the afferents to locus ceruleus is not completely known. In order to determine the relationship between locus ceruleus afferents and TH and TH mRNA expression, we have mapped the topography of TH activity, TH mRNA and neurochemical markers of the cholinergic and GABAergic systems. We used a dual labeled in situ hybridization procedure to localize TH and TH mRNA in brain sections. Rats were treated with a single injection of reserpine (7.5 mg/Kg/s.c.) or vehicle. Three days after injection, the animals were sacrificed and the brain rapidly removed and frozen in powdered dry ice. Brain sections were cut at 14mic. Intact TH enzyme was measured in 1mm punches from the locus ceruleus in three successive 200 mic sections. TH mRNA was measured by in situ hybridization in the thin sections, using a 48 oligonucleotide synthetic cDNA probe.

TH mRNA levels differed between subregions of the locus ceruleus. After reserpine, TH activity and TH mRNA increased in the different subregions of the LC. The regionally selective change in TH and TH mRNA suggested that those neurotransmitters that are highest in the affected regions play a major role in regulation of TH gene expression.
393.9
CHRONIC ANTIDEPRESSANT REGULATION OF c-Fos EXPRESSION IN RAT CEREBRAL CORTEX. S.M. Winston*, M.D. Haywood, E.J. Nestler, and R.S. Duman. Laboratory of Molecular Psychiatry, Dep't of Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Acute seizures and other stimuli cause a rapid induction in neurons of the nuclear protein c-fos, an effect which may underlie some long-term adaptations to stress and to acute neuronal activation. In the present study, we examined the influence of chronic electrophoretic seizures (ECS) on the regulation of c-fos induction in rat cerebral cortex. We found that ECS increased the induction of c-fos mRNA by a 2nd ECS, blocked, in agreement with earlier findings, but by a 1st c-fos mRNA could be re-induced by a second ECS, indicating that the responsiveness to ECS can be fully restored after 1 d. However, when rats received chronic (daily) ECS, c-fos induction in response to the most recent ECS showed a time-dependent decrease. This effect was maximal after 7-10 d of chronic ECS, when the induction of c-fos mRNA, as well as of Fos protein, by the most recent seizure was completely blocked. Chronic ECS similarly blocked the induction of c-fos mRNA 4 hr after acute ECS, but not at control levels after chronic ECS.

In preliminary studies, chronic (18 d) imipramine administration enhanced acute ECS induction of c-fos mRNA in cerebral cortex by approximately 2-fold. Chronic imipramine regulation of c-fos expression could reflect an altered responsiveness of cerebral cortical neurons to functional activity and may be related to chronic adaptations in these neurons thought to be critical to antidepressant efficacy.

393.10

Chronic administration of many types of antidepressants down regulates the postsynaptic beta-adrenergic receptor in the locus coeruleus (LC), the major noradrenergic nucleus in the brain, to assess the postsynaptic state of the noradrenergic system. Rats were treated chronically with imipramine (IM), tranylcypromine (TCP), electroconvulsive seizures (ECS) or fluvoxamine (FLU) for 3 weeks. Levels of TH immunoreactivity or mRNA were determined in micro-punches of LC and substantia nigra (SN, a dopaminergic nucleus). All 4 treatments resulted in a 50 to 60% decrease in TH immunoreactivity in the LC, while TH levels in the SN were not altered. Moreover, chronic administration of either IM or TCP (FLU and ECS were not tested) decreased the levels of TH mRNA by 50% in the LC, but did not in the SN, while dopamine beta-hydroxylase mRNA levels were not altered in the LC.

The results demonstrate that four distinct classes of chronic antidepressant treatments specifically regulated TH expression in the LC and suggest that such regulation occurs at a pretranslational level. The down regulation of TH in the LC could be a long-term adaptation of the neurons to acute increases in synaptic levels of norepinephrine, although the action of fluvoxamine, a selective serotonin uptake inhibitor, to decrease TH suggests that these treatments may have additional sites of action.

393.11

Cell-cell contact regulates the levels of tyrosine hydroxylase (TH) in bovine adrenal chromaffin cells and in rat pheochromocytoma cells. When rat pheochromocytoma cells (PC12) are cultured at high density (HD) (2 x 10^3 cells/cm^2), TH activity increases over that observed in cells cultured at low density (LD) (1 x 10^3 cells/cm^2). The levels of TH mRNA were determined by dot blot hybridization of total cellular RNA using a 32P-labeled cDNA for TH-mRNA (pTH-3). After 24 hr, the levels of TH mRNA in HD cells are 3-fold higher than those observed in LD cells; after 4 days TH mRNA levels are 6-fold higher in HD cells. When cells are first cultured at HD and then recultured at LD, the elevated levels of TH mRNA in HD PC118 cells can be accounted for in part by an increase in the relative transcription rate of the TH gene.

393.12
TYROSINE HYDROXYLASE IS EXPRESSED IN THE PURKINJE CELLS OF THE ALLELIC MOUSE MUTANTS TOTTINGER AND LEANER. E.J. Nestler and M. Wilson, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The inherited autosomal recessive mutations, toverting (gene symbol: tvt) and leaner (gene symbol: tmm), are functional alleles of a single gene in mice. The toverting mouse mutant exhibits spontaneous absence seizures, focal motor seizures and mild hindlimb ataxia while the congenic mouse mutant displays severe ataxia and focal motor seizures. Because noradrenergic hyperinnervation of the cerebellum, hippocampus and thalamus has been observed in mice treated with MAO A and MAO B inhibitors, the locus of such an abnormality might be found in these regions. In this study, we examined the influence of chronic electroconvulsive seizures (ECS) on the regulation of c-fos induction in rat cerebral cortex. We found that ECS increased the induction of c-fos mRNA by a 2nd ECS, blocked, in agreement with earlier findings, but by a 1st c-fos mRNA could be re-induced by a second ECS, indicating that the responsiveness to ECS can be fully restored after 1 d. However, when rats received chronic (daily) ECS, c-fos induction in response to the most recent ECS showed a time-dependent decrease. This effect was maximal after 7-10 d of chronic ECS, when the induction of c-fos mRNA, as well as of Fos protein, by the most recent seizure was completely blocked. Chronic ECS similarly blocked the induction of c-fos mRNA 4 hr after acute ECS, but not at control levels after chronic ECS.

In preliminary studies, chronic (18 d) imipramine administration enhanced acute ECS induction of c-fos mRNA in cerebral cortex by approximately 2-fold. Chronic imipramine regulation of c-fos expression could reflect an altered responsiveness of cerebral cortical neurons to functional activity and may be related to chronic adaptations in these neurons thought to be critical to antidepressant efficacy.

393.13
THE EFFECT OF AGING ON THE RELATIVE mRNA CONCENTRATIONS OF MONOMOLECULAR CONVEXITY A AND B IN HUMAN BRAIN. Li-Jia Wang*, Nancy Lan*, Rachael Neve*, and Jean C. Shih* (SPRI: J. Ihm) School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033 and Division of Genetics, The Children's Hospital - Boston, MA 02115.

Based on substrate and inhibitor specificities, two types of monomolecular convexity (MOA) A and B, have been defined. Our recent cloning of the genes encoding MOA A and B suggests that the different MOA isoforms reside in their primary structures. By using subfragments of these cDNAs which are specific for MOA A or B, we found that MOA A, but not MOB, exhibit similar patterns of expression in 14 brain regions examined; the relative concentrations of these transcripts are frontal cortex > locus coeruleus > temporal cortex > posterior parietal cortex > occipital gyrus > anterior parietal cortex > hippocampus and thalamus.

Conclusively, with these MOA B catalytic activity increases with age whereas MOA A activity did not change. Interestingly, there is no statistical difference in mRNA levels of MOA A or B in the elderly samples examined. These data suggest that the increase in MOA B catalytic activity is not at the transcription level, but that it results from post-translational modifications of MOA B in aging brains. (Supported by MH30985, MH07976, and Weln professorship)

393.14

MOA concave (MOA C) is an integral membrane enzyme, is involved in the biological inactivation of at least three different transmitter substances: dopamine; norepinephrine, and serotonin, intracellularly, in the brain. Monomolecular convexity (MOA A) mRNA present in human placenta is comprised of two species with approximate molecular size of 2.2 and 4.2 kilobases (Kb). A cDNA clone (pMAO-A1) encoding human MOA A was isolated from a human placental library, pMAO-A1 was 2731 base pairs long and was used to determine the molecular nature for the size heterogeneity of these transcripts. MOA-C1 was derived from the previously published pMAO A cDNA clone (Bach et al., 1988). pMAA USA, 85:4934 at their 5' termini. DNA sequencing indicated that pMAO-A1 contained an additional 1900 nucleotides at the 3' non-coding region. That pMAO-A1 corresponded to the larger mRNA, 4.2 kb long, and hybridization of a unique Hind III/BreI fragment 3' to the first polyadenylation signal (2.2 kb) to the 5' end of pMAO A mRNA. The result suggests that the 2.2 and 4.2 kb mRNA species arise from the alternate use of two polyadenylation signals. We have further isolated and characterized a MOA A genomic clone which included both the coding and noncoding regions. RNA dot blot analysis suggested a single gene may encode MOA A. (Supported by grants from NIAAA and DOH to C.H. and R.H.H.)
It is possible that functional effects of the peptide are mediated through dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA). Cholecystokinin (CCK) coexists with dopamine in many of these neurons. To determine whether haloperidol affects tyrosine hydroxylase (TH) and CCK at the level of transcription, mRNA levels of TH, the rate-limiting enzyme in catecholamine synthesis, and CCK were determined.

Rats were treated with haloperidol (2 X 10 mg/kg i.p. daily) or vehicle for 3 days (acute) or 19 days (chronic) and sacrificed 16 hours after the last dose. In a separate experiment, reserpine (10 mg/kg i.p.) or vehicle was administered in a single dose 24 hours before sacrifice. Brains were frozen-sectioned at 12 µM through the SN-VTA and LC. In situ hybridization was performed using 32P-labeled 48-mer oligonucleotide probes against TH and CCK (gift from W. Scott Young, NIMH). Labeled sections were exposed to Kodak XAR X-ray film and quantitated on an image analysis system.

There was no difference in TH mRNA between control, acute haloperidol, and chronic haloperidol treatments in either the anterior, middle, or posterior VTA. There was no difference in TH mRNA in the SN between the three treatment groups. Similarly, there was no difference in CCK mRNA between control, acute haloperidol, and chronic haloperidol treatments in the anterior, middle, or posterior VTA. There was no difference in CCK mRNA in the SN between the three treatment groups. TH mRNA was analyzed for the LC. There was no difference in mRNA levels between control, acute, and chronic haloperidol groups, although reserpine treatment produced a significant increase in TH mRNA levels in the locus ceruleus (t = 5.938, p < 0.01).

These data suggest that the effects of haloperidol on tyrosine hydroxylase and cholecystokinin in midbrain dopamine neurons do not involve regulation of gene transcription.

**References**

Thompson KA, Widdowson P.S. and Halaris A.E., Dept of Psychiatry, Case Western Reserve Univ, Cleveland, OH 44109.

393.15

**THYROGENIC DOPAMINE AND CATECHOLAMINE mRNA LEVELS IN THE SUBSTRATA NIGRA, VENTRAL SEGMENTAL AREA, AND LOCUS COERULEUS DURING RESERPINE TREATMENT INDUCED BY ACUTE AND CHRONIC HALOPERIDOL ADMINISTRATION.** St. Cottingham, D. Pickel, P. Morgenstern, TK Shigemoto, SM Paul* and PN Crawley. NSB, NIMH, Bethesda, MD 20892.

**393.16**

**RESERPINE INCREASES BOTH TYROSINE HYDROXYLASE AND GALANIN MESSENGER RNA IN THE LOCUS COERULEUS.** M.C. Austin, S.L. Cottingham, S.M. Paul* and L. Crawley. Clinical Neurosciences Branch, NIMH, Bethesda, MD 20892.

Galanin (GAL), a 29 amino acid neuropeptide, coexists extensively with noradrenergic (NE) in perikarya of the locus coeruleus (LC) (Melander, et al. 1986). GAL has been shown to inhibit NE-induced accumulation of cyclic AMP in rat cortical slices (Nishinaka, et al. 1980). Previous studies have documented that administration of LC neurons either pharmacologically or by stressors increases tyrosine hydroxylase (TH) activity (Zigmund, et al. 1974) and TH mRNA (Mallet, et al. 1980). Using in situ hybridization, we have examined the effects of reserpine on both TH and GAL mRNAs in adjacent sections of the LC. Male Sprague Dawley rats were administered with either vehicle or reserpine (2 or 10 mg/kg i.p.) and sacrificed 24 hrs. 3S oligonucleotide probes for TH (48 bases) or GAL (39 bases), synthesized by B. Martin, NIMH, were applied to cortical brain sections (12 um) which were incubated for 24 hrs. Kodak XAR X-ray film exposed by the labeled sections was quantitated using a computerized densitometry image analysis system (W. Rasband, NIH). Reserpine significantly increased both TH and GAL mRNA in LC neurons. These results confirm previous reports that reserpine increases TH mRNA concentrations in the LC. In addition, the finding that reserpine also increases GAL mRNA raises the possibility that a common reserpine-sensitive mechanism regulates the expression of both the tyrosine hydroxylase and galanin genes.

Recently we reported on the intriguing structural similarities of glutamic acid based CCK antagonists (CR-1409) and the benzoazepinlike CCK antagonist L-364,719. In our search for novel CCK receptor antagonists we have attempted to include the weak glutamic acid based CCK antagonists (CR-1409) and the development of potent and selective glutamic acid based hybrid antagonists. Utilizing a similar approach to that which enabled the discovery of these compounds and provide preliminary in vitro characterization of these derivatives. A prototypical example of this class is A-67,396 (IC50 = 30 nM). With these new agents it may be possible to further probe the peripheral type A CCK receptor.

BINDING CHARACTERISTICS OF ANGIOTENSIN II AND III TO NG108-15 CELLS. M.D. Carrithers, S. Masuda*, K.A. Koide*, and J.A. Weyhenmeyer, Program in Neural and Behavioral Biology and College of Medicine, University of Illinois, Urbana, IL 61801.

Previously we demonstrated that NG108-15 cells differentiated by treatment with 1.5% dimethyl sulfoxide (DMSO) and 0.5% fetal bovine serum for three to four days express both low and high affinity sites for angiotensin II (ANG II). The high affinity site is not expressed on undifferentiated cells, and its binding subunit has an approximate molecular weight of 79 kDa. Bn-AII was synthesized and characterized (Carrithers et al., FASEB J. 3:103-112, 1989). In the present study, we further characterized the binding properties of both the low and high affinity sites. Using I-125-ANG II, Scatchard analysis in the presence of 5 µM bestatin, a peptidase inhibitor, revealed a single low affinity site in undifferentiated cells (Kd = 45 nM; Bmax = 320 fmol/mg protein) and a high and low affinity site in differentiated cells (Kd = 1.95 nM; Bmax = 15% fmol/mg protein; Kd = 5.5 nM; Bmax = 1.676 fmol/mg protein). In competition studies, ANG III specifically displaced high affinity ANG II binding in differentiated cells (Kd = 2.69 nM), but did not displace binding to the low affinity site in either differentiated or undifferentiated cells. We hypothesize that binding to the high affinity site in differentiated cells occurs through the carboxyl-terminus of ANG II or ANG III and that it functions as a true receptor. It is further hypothesized that ANG II binds to the low affinity site through its N-terminus and that this site represents a bestatin-resistant aminopeptidase.

AFFINITY CYTOCHEMICAL LOCALIZATION OF RAT BRAIN ANGIOTENSIN RECEPTORS USING BIOTINYLATED ANGIOTENSIN II (Bn-II). M.S. Brownfield and D.H. Harkness. School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin 53706.

Localization of brain All receptors has previously been studied by quantitative autoradiography which has yielded valuable information on the distribution of receptors in the brain as a whole. However, it does not reveal the location of receptors in the cellular or subcellular levels. Therefore, we have developed a Bn-II probe for use in the affinity cytochemical localization of All receptors at the light and electron microscopic levels.

Bn-II was prepared by incubating synthetic All with an excess of bovine N-terminal hydroyxysuccinimide ester (Bo-NHES). The product was purified using Sephadex G25 chromatography or reverse phase HPLC. Bn-NHES attacks amino groups, and since All has one free amino at the N-terminal position bovin should be linked to the N-terminal residue. This bovin-extended All should retain binding activity since other N-terminal modifications have produced All analogs capable of specific binding to All receptors.

Rat brains were fixed with 2% paraformaldehyde-0.02% picric acid in 0.1 M phosphate buffer, pH 7.5. Cryostat or vibratome sections were first incubated in an avidin-biotin "block" sequence followed by Bn-II (1-100 nM) and avidin-peroxidase Complex. The receptor-Bn-II-A-PO complex was visualized by incubation with DAB+0.6% nickel ammonium sulfate using the glucose oxidase system. Binding of Bn-II was validated by addition of 1-10 µM All in the cytochemical system and by characterization of binding using radioreceptor assays of fresh and fixed brain membrane preps. Staining distribution was limited to nuclei known to contain All receptors. However, receptor localization was shown for the first time at high resolution in association with plasmalemma of neuronal and glial cells. From these studies, Bn-II provides a valuable tool for the high resolution localization of All receptors.
394.9


Retrograde tracers are employed to establish the anatomical projections of single neurons. We have used in vitro receptor autoradiography for angiotensin II (AII) binding to assess possible alterations in functional integrity of dorsal medullary neurons which were bathed with fluorouracil (FG). Fluoro-gold was injected unilaterally into the caudal pole of the nodose ganglion of rats 10–14 days before preparation of the tissue for determination of all binding sites. Fluoro-gold transport was demonstrated by intense labelling of the dorsal motor nucleus of the vagus. Binding data were expressed as the ratio of all binding on the side ipsilateral to injection versus binding on the contralateral (contra) side. Ratio for all binding were determined in rats which had undergone unilateral cervical vagotomy (VGX, 14 d), unilateral nodose ganglionectomy (NGX, 10 d) or with the vagi intact. The table below summarizes the effect of these maneuvers on all binding.

<table>
<thead>
<tr>
<th>Condition</th>
<th>FG</th>
<th>NGX</th>
<th>VGX</th>
</tr>
</thead>
<tbody>
<tr>
<td>% ipsi/contra</td>
<td>98</td>
<td>91.5</td>
<td>53.3</td>
</tr>
</tbody>
</table>

We conclude that after 2 week survival FG does not alter substantially all binding in the dorsomedial medulla. The modest reduction in binding on the side of injection may be attributable to damage of fiber associated with injection of the tracer. These results suggest that FG may be used in combination with receptor autoradiography to determine the efferent projections of neurons whose binding sites are to be studied.

This work supported by NIH grants HL-6835, HL-36988 and HL-38535.

394.11

MODULATION OF ANGIOTENSIN II-INDUCED CYCLIC GMP PRODUCTION IN MURINE NEUROBLASTOMA NIE-115 CELLS BY PERTUSSIS TOXIN. X. Ye and S.J. Hillyer (Spons: M.A. Waczyksp) Dept. of Animal Biology and Biochemistry and Biophysics, and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

In the rat hypothalamic NIE-115 cell line angiotensin II (AngII) stimulated the rapid production of cyclic GMP (cGMP). cGMP production was maximal at 15 sec and continued agonist exposure resulted in diminished cGMP levels. Ang II stimulation was also dose-related with an apparent EC50 of 30 nM, and was maximal at 1 uM. Several AngII related agonists also increased cGMP levels and the rank order of efficacies was Ang II = Ang IV = Ang I = Ang 3-8 = Ang 1-9 (= Ang II). Sar1, Ile8-AngII, a high-affinity antagonist, completely blocked the stimulation elicited by Ang II. Finally, incubation of intact cells for 24 hr with pertussis toxin (100 ng/ml) resulted in an inhibition of subsequent pertussis toxin catalyzed incorporation of [3H]-GTP into a membrane protein of M, 41 KD. Pertussis toxin treatment also significantly attenuated AngII and AngII-induced cGMP production but did not alter the proportion of high affinity AngII receptors, or their regulation by guanine nucleotides. These results suggest that pertussis toxin may be an important intracellular mediator of Ang II actions within neuronal cells, and that pertussis toxin may modulate this transduction process through a mechanism independent of a change in agonist receptor affinity. Supported by NS 23968 and MH 34787.

394.13

IDENTIFICATION OF HIGH AFFINITY [3H]-GASTRIN RELEASING PEPTIDE BINDING SITES ON RAT FOREBRAIN MEMBRANES. P.I. Monroe and S.L. Pedrotti. NOVA PHARMACEUTICAL CORPORATION, BALTIMORE, MD 21224.

In vivo and in vitro pharmacological and biochemical studies suggest that bombesin (BN) and gastrin releasing peptide (GRP) may act as neurotransmitters or neuromodulators in the central nervous system (CNS). In the present study, specific high affinity CNS sites for the putative mammalian analog of BN, [3H]-GRP, were characterized and compared to those previously identified for [3H]-Tyro-3-BN (Moody, et al., Proc. Nat. Acad. Sci. 75, 1978). Rat forebrain membranes were incubated with [3H]-GRP for 30 min at 25°C to determine the number of BP receptors using a Scatchard analysis. [3H]-GRP binding was saturable, with an apparent Kd of 5.6 x 10^-9 M. The number of BP receptors in rat forebrain membranes was 1.6 x 10^13 receptors/mg protein. [3H]-GRP binding was located in the striatum, hippocampus, and cortex. [3H]-TYR-3-BN binding was also observed in the forebrain. The activity of the brain angiotensin system (BAS) has been shown to be altered in several genetic hypertensive models and in response to changes in dietary sodium and to mineralocorticoid administration. In this report we examine the effects of deoxycorticosterone acetate-salt (DOCA-salt) induced hypertension on the expression of the brain angiotensin II (Ang II) receptor. Male Sprague-Dawley rats were uninephrectomized and aged. Animals were then processed for quantitative autoradiography using [125I]-saI, [125I]-sI-Ang II (125I-S-I-Ang II). There was a significant increase in the affinity and Bmax of 125I-S-I-Ang II binding in the solitary-wagal (SVA) area (SVA) from the DOCA-salt rats and an increased Bmax in the DOCA rats. The density of 125I-S-I-Ang II binding was elevated in DOCA-salt rats in a variety of brain regions, including the circumventricular organs, the suprachiasmatic nucleus and the paraventricular nucleus. In contrast, there was no significant change in 125I-S-I-Ang II binding in the anterior pituitary in any treatment group. These results suggest that the BAS may be involved in the expression of DOCA-salt hypertension. (Supported by UGM HL 42585.)
CHARACTERIZATION OF RODENT PITUITARY AND CELL LINE GNRH RECEPTORS EXPRESSED IN RNA-Injected XENOPUS OOCYTES. S. C. Seibt, B. Gilly, S. Munkedam*, P. Hepler, J. Windle, E. Laporte and J. A. Rosenfeld, Fibroblast Center in Neurobiology, Neurology, Psychiatry, Mount Sinai School of Medicine. New York, N.Y. 10029. S. C. Seibt. GNRH receptors were expressed in Xenopus oocytes micro-injected with RNA isolated from rat pituitary and from the αT3 cell line. 3-4 days after RNA injection, cells were recorded by voltage clamp. On exposure to 10^{-7} M GNRH an increase in membrane current was observed in pituitary RNA injected cells and 116±526 nA (n=3) in αT3 RNA injected oocytes. The threshold for obtaining a response after pituitary RNA injection was 3.0±0.7 nM of GNRH (n=2) and 6.0±0.7 nM of GNRH (n=2) in αT3 injected oocytes. The response to GNRH by greater than 90%, while the response to TRH was unchangeable. The threshold concentrations for a response to TRH was 0.1 nM. RNA fractions showed that receptor mRNA's has a sedimentation factor larger than 5S. Studying the molecular mechanism, the response to GNRH was not changed in calcium-free medium. In cells injected with a mixture of brain and pituitary RNA, the response to GNRH was eliminated by intracellulae injection of EGTA whereas the response to GABA was not affected. Ramps studies revealed a reversal potential of the GNRH-generated current of -22±1 mV (n=7) and -25.6±3.3 mV (n=3) in pituitary and cell line RNA injected oocytes respectively, consistent with the chloride reversal potential. The GNRH response is mimicked by intracellular injection of IP3. Normal rat pituitary and αT3 cell line RNA lead to the expression of functional GNRH receptors in Xenopus oocytes. The response is dependent on intracellular but not extracellular calcium and represents a calcium-dependent chloride conductance, suggesting signal transduction by inositol phosphate metabolism.

CYCLIC ANALOGUES OF SOMATOSTATIN DISTINGUISH PHARMACOLOGICALLY AND FUNCTIONALLY DISTINCT SUBTYPES OF SOMATOSTATIN RECEPTORS IN BRAIN AND PITUITARY. K. Ravnor and T. Reisine, Dept. of Pharmacology, Univ. of PA, PHL, 19104. Somatostatin (SRIF) is a tetradecapeptide found widely distributed throughout the CNS where it serves as a neurotransmitter. SRIF induces its cellular effects in brain, including inhibition of adenylyl cyclase activity and modulation of ion currents, through specific membrane receptors. The different physiological actions of SRIF may be mediated by distinct receptor subtypes. The lack of availability of subtype specific compounds has hindered the definition and functional characterization of SRIF receptor subtypes in the brain and pituitary. Employing the SRIF analogues CEP 23996 and MK 678, we have demonstrated the existence of pharmacologically and functionally distinct SRIF receptor subtypes in the rat brain. Within the hypothalamus, [125I]-MK 678 binding is of high affinity and saturable. It is also specific for SRIF receptors, being displaced by low nM concentrations of SRIF and its analogues, but not by other unrelated peptides. (235) CEP 23996 labels a SRIF receptor which is poorly recognized by MK 678 and its structurally similar analogues, even at concentrations as high as 10 uM. These and other data suggest that (125I) CEP 23996 and (125I) MK 678 label distinct SRIF receptor populations in the CNS. In contrast, (125I) CEP 23996 and (125I) MK 678 appear to label a single receptor population in pituitary. Both MK 678 and SRIF inhibit forskolin-stimulated adenylyl cyclase activity in pituitary. While SRIF also inhibits adenylyl cyclase activity in the corpus striatum, a region of brain with a high density of SRIF receptors, MK 678 has no effect on adenylyl cyclase activity in this region. Furthermore, antibodies directed against fragments of the alpha subunit of G proteins partially precipitated the solubilized SRIF receptor and pertussis toxin treatment of AtT-20 cells, a tumor cell line with high expression of SRIF receptors, caused the uncoupling of G proteins from the SRIF receptor and abolished the [125I]-MK 678 labeling of the solubilized SRIF receptor. The [125I]-MK 678 SRIF receptor appears to be tightly associated with GTP binding (G) proteins since all specific [125I] MK 678 binding to the solubilized receptor was blocked by SRIF analogues. Additionally, antibodies directed against fragments of the alpha subunit of G proteins partially precipitated the solubilized SRIF receptor and pertussis toxin treatment of AtT-20 cells, a tumor cell line with high expression of SRIF receptors, caused the uncoupling of G proteins from the SRIF receptor and abolished [125I] MK 678 labeling of the solubilized SRIF receptor. The solubilized SRIF receptor appears to be a glycoprotein since the receptor could be eluted from a WGA column with the sugar TACT. Studies are in progress to determine whether subtypes of solubilized SRIF receptor couple with different G proteins and have different physiological properties. Supported by NIH grant GM 34781 and ONR (14-86-K-0048).

BIOCHEMICAL PROPERTIES OF THE SOLUBILIZED BRAIN SOMATOSTATIN (SRIF) RECEPTOR. H. T. He*, S. Rens-Domiano, S. Borislow* and T. Reisine, Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710. SRIF is a neurotransmitter in the CNS whose physiological actions are mediated by membrane-bound receptors. To gain insight into the molecular mechanisms by which SRIF exerts its actions in the CNS, we have investigated the biochemical properties of the solubilized brain SRIF receptor. Brain SRIF receptors were solubilized with CHAPS and purified with high affinity agarose. The solubilized SRIF receptor appears to be tightly associated with GTP binding (G) proteins since all specific [125I] MK 678 binding to the solubilized receptor was blocked by SRIF analogues. In addition, antibodies directed against fragments of the alpha subunit of G proteins partially precipitated the solubilized SRIF receptor and pertussis toxin treatment of AtT-20 cells, a tumor cell line with high expression of SRIF receptors, caused the uncoupling of G proteins from the SRIF receptor and abolished [125I] MK 678 labeling of the solubilized SRIF receptor. The solubilized SRIF receptor appears to be a glycoprotein since the receptor could be eluted from a WGA column with the sugar TACT. Studies are in progress to determine whether subtypes of solubilized SRIF receptor couple with different G proteins and have different physiological properties. Supported by NIH Grant GM 34781 and ONR (14-86-K-0048).

M-CURRENT NOISE IN RAT SYMPATHETIC NEURONES. D. G. Owen, S. J. Marsh* and D. A. Brown. Dept. Pharmacology, University College London, London WC1E 6BT, U.K. Mucanslur blocks the non-inactivating voltage-activated K current, IK, in rat sympathetic cervical (SCG) neurones (see Brown, 1988). In order to estimate the parameters of single ion channels underlying whole cell M-currents, we applied the technique of noise analysis to muscarine-sensitive currents. Whole cell M-currents were made from fractions of adult rat SCG neurones using a standard extracellular solution and patch pipette solution containing [Ca]o 100 µM free and [Mg]o 1 mM ATP at -40 mV. Currents driven by the voltage clamp before, during and after the application of muscarine were stored on VCR, and subsequently digitised (0.05-5000 Hz) and stored by a PDP 11/23 via CED 502 interface. Spectral analysis was carried out using NOISE (D. Colquhoun). Data acquired at -30 mV was found to lie within the low probability limit and power spectra at this potential were used for analysis of channel noise. Using the analysis of the Lorentzian Functions [eq. $f_{c} = 1.08S / (S^2 + 1.55^2)$, $f_{c} = 32S / (S^2 + 0.02^2)$], Results were consistent with a single M-channel of about 1.6 pS, having at least 3 states. The time constant corresponding to $f_{c}$ is 1.59 ms which is similar to that of macroscopic IK deactivation tails at the same membrane potential (-30 mV). Brown, D.A. (1988) TINS, 11, 294-299.

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POTASSIUM CHANNELS IV
ACETYLCOLINE DOES NOT ALTER POTASSIUM INWARD RECTIFICATION ON XENOPUS SKELETAL MUSCLE IN CULTURE.
F. Moody-Corbett and R. Gilbert. Div. Basic Sci. Memorial University of NF, St. John's, NF, Canada.

Acetylcholine (ACh) causes an increase in the probability of opening inward rectifying K+ channels on heart nodal cells. In this study we examined the effect of ACh on the K+ inward rectifier of skeletal muscle. Nuclear cultures were prepared from Xenopus myotomal muscle and macroscopic whole cell currents were recorded using a patch clamp. The nicotinic ACh receptors and Na+ currents were blocked with bungarotoxin (0.1 µM) and tetrodotoxin (10 µM/g/ml), respectively. The external recording solution contained (mM) 140 NaCl, 5 KCl, 1 MgCl2, 1.2 CaCl2, 10 HEPES (pH 7.4) and the solution in the patch electrode contained (mM) 140 KCl, 1 EDTA, 5 MgCl2, 10 HEPES (pH 7.4). Hyperpolarizing step potentials from resting membrane potential resulted in an inward current that was similar to the K+ inward rectifier on adult skeletal muscle. Application of ACh (0.1-10 µM) to the bath did not alter the amplitude, time course of the activation of inward current on these muscle cells. In contrast to the effects on heart nodal cells, ACh does not alter the properties of the classic inward rectifier. (Supported by MRC, Canada)

395.4
MUSCARINIC AND NORADRENERGIC EFFECTS ON VOLTAGE DEPENDENT POTASSIUM CURRENTS IN CAT BLADDER PARASYMPATHETIC NEURONES.
Eva Vickery* and Patricia Shinnick-Gallagher, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

In cat bladder neurons, acetylcholine and noradrenaline have both excitatory and inhibitory effects. In this study we analyzed the effects of these neurotransmitters on voltage dependent K+ currents using single-electrode voltage clamp. Several types of voltage dependent K+ currents were observed in these neurons. These currents possess similar voltage dependences and pharmacologies as those described previously in other types of central and peripheral neurons and include: a M-current suppressed by Ba2+ (0.3-1.0 mM) and muscarine (10-20 µM); an A-current suppressed by 4-aminopyridine (10 mM); a Ca2+-dependent K+ current sensitive to Co2+ and Cd2+, suppressed by apamin (1.0 µM) and not affected by TEA (2.0-4.0 mM); a Q-current blocked by extracellular Cs+ (3.0 mM) and an anomalous rectifier current which is blocked by Ba2+ and Ca2+ and seems to be Ca2+-dependent. Both muscarine (3-10 µM) and noradrenaline (10 µM) induced a net outward current and enhanced anomalous rectification. Muscarine (10 µM) suppressed I_L and I_KO but noradrenaline appeared to have no effect on either of these currents. (Supported by NS16228)

395.5
CYCLIC AMP DEPENDENT CATION CURRENT IN DISASSOCIATED BULLFROG SYMPATHETIC AND PRIMARY AFFERENT NEURONS. T. Takimura, N. Hamanaka, T. Mitohara, K. Tsukui, N. Shibatani*, T. Akasu*.

DEPT. OF PHYSIOLOGY, KURUME MEDICAL FACULTY, KURUME, JAPAN.

A hyperpolarization-activated sodium/potassium current (gH) was recorded from cultured bullfrog sympathetic neurons in the whole-cell configuration. gH activated between -130 and -80 mV (50% activation between -90 and -95 mV). A hyperpolarizable form of ATP in a pipette solution was necessary for gH. Both application of ATP (1 µM) had no effects on gH while the drug selectively increased the N-current (I_N). The maximum conductance of gH was increased by intracellular "loading" of cyclic AMP (3-10 µM), or bath application of forskolin (10 µM). A forskolin sensitive cyclic AMP analogue (IOM) and IBMX (0.1-1 mM) increased gH. Open close kinetics of gH showed a deactivating shift when it was facilitated by intracellular cyclic AMP, gH but not gH was depressed by C-Kinase inhibitor phorbol 12-myristate 13-acetate (3 µM). The amplitude of gH was approximately halved by protein kinase inhibitor H-8 (3 µM). Essentially the same results were obtained from cultured dorsal root ganglion cells. It is concluded that a voltage-gated cation current is also controlled by basal activity of cyclic AMP presumably via protein kinase-A. Supported by the Naito Foundation (87-128).

395.6

Venom toxins have been useful in the characterization of ion channels by the utilization of their specific channel binding properties. In this work we tested the effects of the K channel blockers Pandinus imperator scorpion venom, charybdotoxin (CTX), and apamin on frog skeletal muscle voltage dependent K-currents (I_KDR) and inward rectifier K-currents (I_KIR).

Bullfrogs were decapitated and pithed, and the semimembranosus muscle was dissected and placed in frog Ringer's. Single muscle fiber strips were mounted in the Hiline-Campbell vamise gap chamber. The fiber ends were cut in 80M K-EGTA to maintain internal calcium concentrations at low levels to preven activation of contraction and Ca-activated K currents. Voltaic records were obtained in either 0.1% BSA frog Ringer, pH 7.4 to test for I_KDR or in 0.1% BSA K-methylsulfonate acid, pH 7.4 solution to test I_KIR. Holding potentials were 40 mV and were maintained in 120M KMSA. Currents were measured in the voltage clamp mode in response to both steps and ramps of command voltage. Untreated Pandinus venom (200 µg/ml) blocked both I_KDR and I_KIR but had no significant effect on I_KDR. I_KER, neither heat-inactivated (200 ug/ml) nor dialysed (500 µg/ml) venom had any significant effect on I_KDR. 200 µg/ml a catalase of Pandinus venom, had a similar but smaller effect than whole venom on I_KDR. CTX (200uM) and apamin (500uM) two blockers of different Ca-activated potassium channels, had no significant effects on frog skeletal muscle K currents, I_KDR or I_KER. Previously it was believed that Pandinus venom did not block I_KDR of frog skeletal muscle, and that all the effects seen were due to zinc contained in the venom. Here we show that whole Pandinus imperator venom can have effects on I_KDR of frog skeletal muscle. (NIH AR-34766, NS07300)

395.8

Apamin, a bee venom neurotoxin, has been shown to selectively block one class of Ca2+-activated K channels, EGK(Ca). Intracellular recordings were made from locust corpus locularis (LC) neurons in completely submerged brain slices under current- and voltage-clamp conditions. Bath superfusion of 100-500 µM EGK(Ca) had no significant effects on the single spike AHP and the post-spike hyperpolarization (FSH) which follows a train of action potentials. Apamin reduced the magnitude of the late part of the single spike AHP with no change in its peak amplitude. In most LC neurons, FSH elicited by a train of action potentials consisted of two distinct phases which could be fitted by a biexponential function. The initial, fast phase had a decay constant one order of magnitude smaller than that of the late, slow phase. Both phases were abolished in low Ca2+ medium and by Ca-channel blockers, which suggests that they are mediated by an activation of EGK(Ca). Apamin abolished the initial, fast FSH and exaggerated the late component. Apamin also increased the number of spikes evoked by a long depolarizing pulse. These data suggest that apamin-sensitive EGK(Ca) underlies the late part of the single spike AHP and fast phase of FSH, and is involved in regulation of repetitive firing in LC neurons. Grant support: PHS AA-5846 (S.A.S.)
395.9

EFFECTS OF K CHANNEL BLOCKERS ON ATP-SENSITIVE, CA-ACTIVATED, AND DELAYED RECTIFIER K CHANNELS IN INSULIN-SECRETING HIT CELLS. S. Fatheree and D.L. Cook*. Dept of Physiology/Biophysics and Medicine, Univ. of Wash. and VA Medical Center, Seattle, WA, 98108.

Whole-cell and excised outside/out patch clamp techniques were used to study the effects and mechanisms of tetraethylammonium (TEA) and quinine on Ca-activated K (K(Ca,V)) and ATP-inhibited K (K(ATP)) channels and delayed rectifier current in HIT cells. HEPES-buffered (pH 7.2) solutions containing 120 mM NaCl, 2 mM KCl, 12 mM CaCl2, 10 mM HEPES (pH 7.4) were used. TEA reversibly and rapidly decreased the open channel conductance of K(Ca,V) and K(ATP) channels, and reduced del-rectifier current. It was more effective on K(Ca,V) (Kd=200µM, n=8) than on K(ATP) (Kd=200µM, n=8) and delayed rectifier current (Kd=200µM, n=3). TEA did not affect gating of K(Ca,V) and K(ATP) channels. Quinine decreased open channel conductance of K(Ca,V) (Kd=500µM, n=6) and reduced delayed rectifier current (Kd=600µM, n=5). On the other hand, quinine did not affect K(ATP) single channel conductance but did reduce the frequency of channel opening (Kd=100µM, n=7).

Conclusion: Low doses (50µM) TEA provides relatively specific block of K(Ca,V) (Kd=200µM) whereas high doses (500µM) of quinine is relatively specific for K(ATP) channels. Quinine inhibits K(ATP) by interfering with channel opening while it inhibits K(Ca,V) channels by directly blocking K flow through open channels.

Supported by Juvenile Diabetes Foundation grant #388029 and the Veterans Administration.

395.10

VOLATILE ANESTHETICS BLOCK A POTASSIUM CHANNEL IN NOCICEPTORS. M.B. Mactier and D.L. Tanelian. Dept of Anesthesia, Stanford Univ. Sch. of Medicine, Stanford CA 94305.

Although general anesthetic actions on free nerve endings are not thought to contribute greatly to anesthesia, few detailed studies are available to support this. The present study investigated the effects of different concentrations of halothane and isoflurane on Ca and C fiber nociceptors using a new in vitro preparation from rabbit cornea. The anesthetics produced concentration and time dependent changes in excitability and peripheral discharge activity. Increased discharge frequency was produced by low concentrations (0.5-0.8 X MAC), burst discharges occurred at > 1 MAC and depression of discharge was observed at concentrations > 2.5 x MAC. The potassium channel blocker 4-AP (0.25 µM) produced a similar profile of excitation and bursting. TEA, Ba, GTP and 5-HT were not effective.

The results indicate that clinically relevant concentrations of volatile anesthetics excite free nerve endings by depressing a potassium current. In addition, the anesthetics produced a decrease in spike amplitude. These effects on peripheral fibers would be expected to produce the central depressant actions of the anesthetics by increasing excitatory afferent input to the CNS. Supported by the American Cancer Society and a Parker B. Francis Investigatorship in Anesthesiology.

396.1

MODULATION OF FIRING BEHAVIOR AND AFTERHYPERPOLARIZATIONS IN SENSORMOTOR CORTEX BY SEROTONIN (5HT). R.C. Feghali, Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN, 38112.

Neocortical neurons were studied in vitro in brain slices from pericruciate cortex of cats and the sensorimotor region of rat cortex. Neuronal firing behavior and responses to 5HT were similar for neurons from the two species. Bath application of 5HT (10-100 µM) resulted in (i) an initial hyperpolarization (brief and variably present), (ii) a prolonged depolarization which was usually associated with an increase in input resistance, and (iii) a reduction in the slow afterhyperpolarization seen following sustained repetitive firing. In the presence of 5HT, cortical neurons fired faster in response to a given current injection, exhibited less spike-frequency adaptation, and showed less habituation to repeated suprathreshold stimuli. Single electrode voltage clamp suggests that the slow afterhyperpolarization reduction was due to a reduction in the slower Ca2+-dependent K+ current. 5HT also appears to reduce the duration of the medium afterhyperpolarization seen following a single action potential. Several agonists and antagonists were studied to deduce the 5HT receptor subtypes involved in the above responses.

396.2

MODULATION OF POTASSIUM CURRENTS IN CULTURED HUMAN CORTICAL NEURONS. S. E. Guggino (1, 2), G. Bonnett (3), L. Hester (2), and S.R. Snyder (2). Depts of Medicine (1), Neuroscience (2), and Neurology (3). The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Whole cell currents from human cortical neurons were assayed for changes with growth conditions. Neurons isolated from a case of childhood megalencephaly were passed by trypsinization, plated for 2 days then differentiated with nerve growth factor. Isomethylbutylxanthine (IBMX) and dibutyryl cyclicAMP (dibut cAMP) for 2 days. Cells grew long branched processes, expressed neurofilament protein, and neuron specific enolase. Whole cell currents were measured using patch pipettes containing in mM: 5 MgATP, 1 KEGTA, 40 KC1, 1 CaCl2, 1 MgCl2, 20 glucose and NaHepes pH 7.4. Under these conditions the major current was a voltage-dependent outward current which did not inactivate during 250 ms depolarizing pulses and increased 2x when the holding potential (HP) was shifted from -60 mV to -100 mV. At HP of -100 mV and depolarization to +50 mV the outward current was 800 pA. With 0.5 mM IBMX and 0.5 µM dibut cAMP in the bath, a new outward current appeared which showed voltage-dependent inactivation. With HP -100 mV and depolarization to +50 mV this outward current was 120 pA and inactivated within 50 ms. We conclude that rapidly inactivating currents are modulated by increased intracellular cAMP.

396.3


CGRP, an 37 amino acid peptide identified in spinal cord of vertebrates and in motor nerve endings of mammalian neuromuscular junction, is known to activate the biogenesis of cyclic AMP, the physiological activator of protein kinase A. In order to investigate whether CGRP may similarly activate voltage-dependent channels function, experiments were performed on neocortical neurons from 15 - day rat embryos grown in dissociated cell culture for 1 - 6 weeks. Outward currents evoked by depolarization commands from - 80 mV holding potential exhibited a recovery of cyclic AMP inhibited (konopka et. al. 1989). We demonstrate that following substitution of Mn+ or Mg2+ for extracellular Ca2+, the amplitude of the galanin-induced hyperpolarization is decreased reversibly. Also, the galanin-induced hyperpolarization was not reduced in the presence of Mn+ or Mg2+ containing solution, the galanin-induced hyperpolarization decreased progressively. The present study indicates that rapidly inactivating currents were not inactivated during 250 ms depolarizing pulses and increased 2x when the holding potential (HP) was shifted from -60 mV to -100 mV. At HP of -100 mV and depolarization to +50 mV the outward current was 800 pA. With 0.5 mM IBMX and 0.5 µM dibut cAMP in the bath, a new outward current appeared which showed voltage-dependent inactivation. With HP -100 mV and depolarization to +50 mV this outward current was 120 pA and inactivated within 50 ms. We conclude that rapidly inactivating currents are modulated by increased intracellular cAMP.

396.4

GALANIN ACTIVATES A CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN MUDPUPPY PARASYMPATHETIC NEURONS. K.N. Konopka and R. L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

The neuropeptide galanin initiates a slowly developing and longlasting hyperpolarization of neurons in the mudpuppy (Necturus maculosus) cardiac ganglion by activating a membrane K conductance. (Konopka et al., J. Physiol. 410: 107, 1989). We demonstrate in the present study that following substitution of Mn2+ or Mg2+ for extracellular Ca2+, the amplitude of the galanin-induced hyperpolarization is decreased reversibly. Also, the galanin-induced hyperpolarization was not reduced in the presence of Mn2+ or Mg2+ containing solution, the galanin-induced hyperpolarization decreased progressively. The present study indicates that rapidly inactivating currents were not inactivated during 250 ms depolarizing pulses and increased 2x when the holding potential (HP) was shifted from -60 mV to -100 mV. At HP of -100 mV and depolarization to +50 mV the outward current was 800 pA. With 0.5 mM IBMX and 0.5 µM dibut cAMP in the bath, a new outward current appeared which showed voltage-dependent inactivation. With HP -100 mV and depolarization to +50 mV this outward current was 120 pA and inactivated within 50 ms. We conclude that rapidly inactivating currents are modulated by increased intracellular cAMP.
were affected by a change in pHQ, being increased at

**ACTIVATION OF K+ CURRENTS IN CULTURED SCHWANN CELLS IS CONTROLLED BY EXTRACELLULAR PH**

R. Nopp, B. F. Lax and M. Schachner and K. Kettman
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K+ currents recorded from cultured Schwann cells of mouse dorsal root ganglia were sensitive to changes in extracellular pH (pHj). Currents were activated at potentials more positive than -50 mV, which is close to the resting membrane potential, and current amplitudes were affected by a change in pHj, being increased at alkaline and decreased at acidic pHj. Analyzing the time course of current activation at different pHj values indicated that the 

**REGULATION OF SCHWANN CELL K CHANNELS DURING INITIAL STAGES OF MYELINGENESIS G.F. Wilson**

Department of Neurophysiology, University of Wisconsin, Madison, WI 53706.

In adult mammalian Schwann cells, ion channel expression is related to myeligenic phenotype. K+ currents are detectable at the soma of non-myelinating cells but not myelinating ones. When Schwann cells express K+ currents, a developmental change occurs in K+ channel expression when the cell begins to form myelin. We studied K+ currents in Schwann cells using two techniques: patch clamp recording and amperometry. We found that both techniques could detect K+ currents in Schwann cells. The K+ currents were activated at potentials more positive than -50 mV, which is close to the resting membrane potential, and current amplitudes were affected by a change in pHj, being increased at alkaline and decreased at acidic pHj. Analyzing the time course of current activation at different pHj values indicated that the pH-sensitive currents were not located in the channel pore. Under the assumption that nerve activity in the peripheral nerve is associated with pHj changes, as demonstrated for the optic nerve, the pH-sensitive K+ channel of Schwann cells could serve to facilitate the spatial buffering of extracellular K+.
INTERACTION OF GONADAL STEROIDS WITH GABA<sub>a</sub> RECEPTORS IN FRESH SLICES OF MEDIOBASAL HYPOTHALAMUS AND CEREBRAL CORTEX. W. Jacobson, D. Markey, F. Van Huylenbroeck, M. Cyngler, N. MacLusky and C. Shaw. Division of Reproductive Sciences, Toronto General Hospital and Department of Obstetrics, University of British Columbia.

GABA is the most ubiquitous neurotransmitter in the brain. It has been proposed that it plays an important role in the regulation of lipids release and ultimately gonadotropin secretion. We have examined the effects of low dose regimens of gonadal steroids on ovary-dependent females on GABA<sub>a</sub> receptors in slices of mediodasal hypothalamus (MBH) and cerebral cortex. Female Sprague-Dawley rats which had been ovarectomized for 7 days were treated with estradiol benzoate (EB, 5 μg) or vehicle alone at 1000 hrs on day 6. On day 2, animals were treated with estradiol benzoate (EB, 5 μg) or vehicle alone at 1000 hrs. Animals were sacrificed at 1100 hrs, and GABA<sub>a</sub> receptor binding in slices of fresh brain tissue was determined using the GABA<sub>a</sub> antagonist [³H]SR-95531. Treatment with EB alone resulted in a decrease in the amount of [³H]SR-95531 bound in MBH. Subsequent treatment with progesterone resulted in a further decrease in [³H]SR-95531 binding in the MBH of a magnitude similar to that seen with EB treatment alone, in slices of cerebral cortex. Binding in binding with low concentrations of [³H]SR-95531 showed an identical pattern; however, when a high concentration of the radioligand was used the differences in binding between the three groups disappeared. We suggest that changes in behavior and in gonadotropin release normally seen in P-treated, EB-primed animals may be reflective, in part, of a diminution in the inhibitory influence of GABAergic neural circuits (Supported by a grant from the MRC of Canada to MC and from Wyeth Pharmaceuticals to WJ).


Certain metabolites of progesterone markedly potentiate neuronal responses to GABA. In the present study we show that in cultures of chick spinal cord neurons progesterone itself also enhances GABA-induced chloride currents and antagonizes those induced by glycine.

Using whole-cell recording methods cells were voltage-clamped at -70 mV. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipettes. Progesterone (10-100 μM) rapidly and reversibly potentiated responses to 3 μM GABA but reduced responses to 50 μM GABA. These effects on GABA and glycine responses were dose-dependent, with EC₅₀s of 26 and 15 μM, and maxima of +157% and -57%, respectively. These results not only provide an interesting distinction between chloride-mediated GABA and glycine responses, but also suggest that endogenous progesterone may differentially modulate the inhibitory actions of these two neurotransmitters.

EFFECT OF STARVATION ON GABA CENTRAL AND PERIPHERAL BENZODIAZEPINE RECEPTORS. M. Bidder*, R. Neuman*, F. Fares* and M. Gavish. Dept. of Pharmacology, Technion-Fac. of Med., Haifa, Israel.

The effect of 5 days of food deprivation and refeeding for 5 days on GABA receptors, central benzodiazepine receptors (CBR), and peripheral benzodiazepine binding sites (PBS) was evaluated in adult female Sprague-Dawley rats in the estrous phase. Five days of food deprivation caused a significant reduction (20%) in body weight compared with deprived rats: (212 g vs. 161 ± 7 g; p < 0.01). Starvation induced a significant down-regulation (30%; p < 0.01) in PBS in the kidney. The alteration returned to normal values following 5 days of feeding. Ovarian PBS were not affected by food deprivation. [³H]PK 11195 is the receptor ligand as well as the pituitary gland was unchanged. Starvation did not affect CBR binding to cerebral cortex, but it induced a significant decrease (35%; p < 0.01) in [³H]muscimol binding in this brain region. These results indicate that starvation stress affects GABAergic and benzodiazepineergic systems.


Acute administration of barbiturates or benzodiazepines (BDZs) and steroids enhance GABA-mediated responses. Chronic BDZ exposure reduces allosteric coupling between sites on the GABA<sub>a</sub> receptor. Here we report the effects of chronic barbiturate and steroid treatment of cultures derived from embryonic chick brain. Cultures were treated with barbital (1 mM), pentobarbital (200 μM), progesterone metabolites (5-3'- and 5-3'-pregnan-ol-20-on; 10 μM) or β-estradiol (10 μM) for 48 h. Allosteric interactions were measured by reversible binding with 11M [³H]flunitrazepam in the presence and absence of 10 μM GABA. Both barbiturates and steroids reduced allosteric coupling between GABA and BDZ recognition sites, i.e. GABA's ability to enhance flunitrazepam binding was reduced by 50-50%. Barbiturates were more potent than barbiturates. Saturation analysis showed no change in receptor number. Direct enhancement of BDZ flunitrazepam binding by either barbiturates or steroids was abolished following chronic treatment. These results suggest that brain neurons in primary culture exhibit dynamic cellular regulatory responses to both barbiturates and steroids.
PHARMACOLOGICAL DIFFERENCES BETWEEN GABA RECEPTORS OF
Buckingham*, J. Rauh* and D.B. Sattelle. AFRC Unit, Dept.
of the vertebrate peripheral benzodiazepine binding site.
pharmacological properties differ. Electrophysiology,
a benzodiazepine binding site more closely resembling that
INSECTS AND VERTEBRATES. S.C.R. Lummis*, S.D,
vertebrate GABA receptor Cl" channel molecules at the
Selected steroids with IC.İ O ĩ , of 10-1000nM against
conclude that there are differences between insect and
S
antibody.

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THE role of GABA as a neurotransmitter in the hypothalamus is a
subject of considerable investigation. Especially interesting are putative
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embyronic hypothalamus indicate that there have been substantial populations of cells
immunoreactive for tyrosine hydroxylase (TH) and also that the majority of neurons are immunoreactive for GABA. We have also shown that the majority of neurons in the hypothalamus express GABA-benzodiazepine (PBZ) receptor
immunoreactivity. In this study, we wanted to ascertain whether TH-containing cells express the GABAr. Cultures were fixed in 4% paraformaldehyde (PFA)
elements were done on slices of the nucleus. The release of [3H]GABA was induced by high (15 mM) K" inhibited GABA release (IC50 = 35 nM). Both diazepam and pentobarbital caused a parallel shift to the left of the dose-response curve of the muscimol inhibition of the release. The monoclonal antibody (62-3G1) also shifted to the left the dose-response curve. The shifting of the curve by both diazepam and the antibody was blocked by the selective antagonist of the benzodiazepine receptors, Ro15-1788. The results suggest that the monoclonal antibody activates the benzodiazepine receptors of the GABA-A/benzodiazepine receptor complex increasing the affinity of the presynaptic GABA receptors for GABA agonists.

MODULATION OF CELL MITOTICITY BY RO 5-4846 AND
We have used the Nb2 node lymphoma cell line as a model system in which the function of the PBZR may be studied. These cells possess high affinity PBZR sites that are dependent on prolactin (PRL) for mitogenesis. Ro 5-4846 (agonist) and PK11195 (antagonist) bind with high affinity to the peripheral benzodiazepine receptor (PBZR). We report that Ro 5-4846 and PK11195 modulate the mitogenic activity of PRL in Nb2 cells. Ro 5-4846 and PK11195 promoted PRL-directed cell proliferation while at 10^-9M only Ro 5-4846 inhibited the PRL effect. Further, Ro 5-4846 enhanced PRL-stimulated ODC activity at 10^-9M while 10^-8M inhibited the PRL effect. Next, studies were done to determine the effect of PK11195 on the Ro 5-4846 modulation of PRL. At 10^-9M, PK11195 blocked the PRL modulatory actions of 10^-9M and 10^-8M Ro 5-4846. In addition, Ro 5-4846 at 10^-8M blocked the ability of 19-MK PK11195 to promote the mitogenic action of PRL. Finally, the presence of both agents at 10^-7M did not cause a greater effect on PRL-dependent mitogenesis than that shown by the agents individually. These data suggest that both ligands act on the same PBZR sites. Supported by Ariz. Dbl. Contr. Comm.(YG-9290)(HEL) and Finn Pdm. 042-100-099-86(HEL & DIHR).

EXPRESSION OF GABA-RECEPTOR-LIKE IMMUNOREACTIVITY BY
TYROSINE HYDROXYLASE CONTAINING HYPOTHALAMIC NEURONS IN
The role of GABA as a neurotransmitter in the hypothalamus is a subject of considerable investigation. Especially interesting are putative interactions between GABA and dopaminergic neurons. Our previous immunocytochemical characterization of dissociated neurons from the embyronic hypothalamus indicate that there have been substantial populations of cells immunoreactive for tyrosine hydroxylase (TH) and also that the majority of neurons are immunoreactive for GABA. We have also shown that the majority of neurons in the hypothalamus express GABA-benzodiazepine (PBZ) receptor
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CENTRAL AND PERIPHERAL GABA RECEPTORS IN THE

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PHARMACOLOGICAL DIFFERENCES BETWEEN GABA RECEPTORS OF

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Biochemical and pharmacological characterization of AHR-1474 (5-chloro-N,N-dimethyl-2-(4-methylphenyl)-3H-imidazo[4,5-b]pyridine-3-acetamide) indicates that AHR-1474 (2.5mM) binding in rat cerebral cortical membranes preparations were shallow with Hill coefficients of 0.634 and computer analysis indicated two sites with K_values of 41 and 1400 nM. As shown in the AHR-1474 competition for [3H]flunitrazepam (PBZ) binding to the BZ receptor in rat cerebellum revealed a single site with a K_value of 56 nM indicating that AHR-1474 had a 30 fold selectivity for the BZ1 receptor. GABA and bicuculline shift ratios of 2.75 and 2.65 indicated that AHR-1474 behaved as a full agonist and scathead analysis indicated that AHR-1474 was a competitive inhibitor of BZ receptors. In vivo studies showed that AHR-1474 was an effective anticonvulsant as indicated by its ability to inhibit maximal electroshock-induced (ED90 = 9.1 mg/kg/p.o) and subcutaneous Metrazol-induced (ED90 = 2.98 mg/kg/p.o) convulsions in mice. AHR-1474 was also shown to be a potent anxiolytic as indicated by the minimum effective doses in the Vogel (3.16 mg/kg/p.o) and in the light/ dark exploratory (0.10 mg/kg/p.o) behavioral tests in mice. Usual blockade of morphine-induced Straub Tail (ED90 = 0.87 mg/kg/p.o) and the rotordr motor performance (ED90 = 17.8 mg/kg/p.o) tests, AHR-1474 was shown to have less muscle relaxant activity than diazepam. Using values derived from the behavioral and Straub Tail tests, AHR-1474 had a 75 times better anxiolytic/muscle relaxant therapeutic index than diazepam.

GABA A RECEPTOR ALTERATIONS FOLLOWING PERNIAL EXPOSURE TO DIAZEPAM. R.J. Green, J.D. Elsworth and R.H. Rott. (Sponsor: A. Rasmusson). Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Studies examining the relationship between perinatal exposure to diazepam (DZ) and subsequent alterations in central benzodiazepine receptors have yielded inconsistent results. We have examined the effect of perinatal exposure to DZ on the low affinity GABA A receptor in the adult rat. Rats were exposed to DZ from embryonic day 8 to postnatal day 7 (P7). Animals were sacrificed at P90 and several mesolimbic/epileptic brain regions were removed. The binding of the GABA antagonist, [3H]bicuculline methyl chloride (BMC), to P3 membranes in potassium phosphate/thiocynate buffer was measured. A significant decrease in BMC binding was found in cingulate cortex membranes from animals exposed to DZ, relative to untreated controls. In addition, the ability of GABA to displace BMC binding was significantly reduced in hypothalamic membranes from DZ-exposed subjects. Our results suggest that some of the behavioral and biochemical alterations previously seen in animals perinatally exposed to DZ may be associated with an alteration in the GABA A receptor. This work was supported in part by MH 14092 and the Abraham Rubinstein Research Facilities of the Connecticut Mental Health Center.

DECREASED PERIPHERAL BENZODIAZEPINE BINDING CAPACITY IN DEPRESSED PATIENTS. D.L. Diorio, S.A. Weiner, B.E. Suranvi-Cadolte and D.L. Diorio. Dep. of Exp. Biology, New York University. We have previously shown that chronic treatment with 6.7 mg/kg/50% 7,8 dihydroxyflavone (FGF) produces biochemical and behavioral changes in rats which is associated with a down-regulation of the GABA-A receptor complex (Brain Res. 291: 173-177). We have also shown that FGF can modulate the role of the GABergic system in the development of chemical kindling. In order to evaluate the role of FGF on the sensitization of the rats to convulsions and the function of the GABA-A receptor, we have investigated the ability of FG to sensitize rats to kindling with P2Z, a blocker of the GABA-coupled chloride ionophore. Repeated administration of FG (4235.9+477.3) mg/kg/p.o., 3 times a week for 10 weeks resulted in the kindling of convulsions appeared by the 2nd-3rd week of treatment and 90-100% of the rats were sensitized to kindling with P2Z, a blocker of the GABA-coupled chloride ionophore. Supported by FCAR & OCI .

KINDLING PRODUCED IN RATS BY PENTYLENETETRAZOL (P2Z) IS ASSOCIATED WITH A DECREASE IN THE GABA-STIMULATED 35CI- UPTAKE IN THE CEREBRAL CORTEX. M.S. Abraham, R. Lungan*, M. Orlandi*, P. Iaccea*, E. Faradada and G. Biggio. Dep. of Exp. Biology, New York University. We have previously shown that chronic treatment with 6.7 mg/kg/50% 7,8 dihydroxyflavone (FGF) produces biochemical and behavioral changes in rats which is associated with a down-regulation of the GABA-A receptor complex (Brain Res. 291: 173-177). We have also shown that FGF can modulate the role of the GABergic system in the development of chemical kindling. In order to evaluate the role of FGF on the sensitization of the rats to convulsions and the function of the GABA-A receptor, we have investigated the ability of FG to sensitize rats to kindling with P2Z, a blocker of the GABA-coupled chloride ionophore. Repeated administration of FG (4235.9+477.3) mg/kg/p.o., 3 times a week for 10 weeks resulted in the kindling of convulsions appeared by the 2nd-3rd week of treatment and 90-100% of the rats were sensitized to kindling with P2Z, a blocker of the GABA-coupled chloride ionophore. Supported by FCAR & OCI .

GABA MEDIATED CHLORIDE FLUX IS HOT ALTERED DURING MAXIMAL BENZODIAZEPINE (BZ) WITHDRAWAL (WD). Norman. R. Boisse, Yu Xie, and Gary M. Samorajski. S c h o o l 0 f  Pharmacology, Northwestern University, Boston, MA 02115.

Chronic BZ anxiolytic-sedative-hypnotics can induce physical dependence, withdrawal and_bw. If_BD2M are unknown. Spinal cord electrophysiological studies revealed GABA-mediated pre-synaptic inhibition is profoundly reduced during maximal withdrawal (WD) in a rebound way (JPET 222): 664, 1994) supporting a GABA hypoeffectiveness hypothesis. To test whether the GABA receptor-channel complex is a locus for GABA transmission deficits, brain microsac GABA stimulated CI flux (Harris & Allan, 1986) was evaluated during WD. Rats got chloridiazepoxide (CDP) 150 mg/kg, b.i.d. or H2O (controls) for 5 weeks: 3 1/2 days, at max WD flux was found. There was no change in the GABA dose response. CDP, centrally acting pentobarbitral increased GABA flux 5% and 46% in both groups. Therefore, GABA receptor-C1 channel coupling is not altered in WD. Disparity with in situ spinal reflex study suggests endogenous modulators of the GABA receptor complex, intact synaptic transmission and/or reduced B2G release may play the role in WD expression (BRSF 507 RR 05830-09).

DISTRIBUTIVE STIMULUS EFFECTS AFTER MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) AFTER CEREBRAL INFARCTION IN RATS. C.A. Sannerud and R.R. Griffiths. Depts. of Psychiatry and Neurosciences, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Since the drug discrimination procedure has been shown to provide a highly specific assessment of the pharmacological actions of the benzodiazepines (BZ), a drug discrimination paradigm was used to investigate central sites of BZ activity. Male Long-Evans hooded rats were trained to discriminate 0.32 mg/kg MDZ i.p. vs. no drug (ND) in a 2:1 lever discrimination procedure. Rats were implanted with bilateral intracranial cannulae in the basolateral amygdala and lateral ventricles or basolateral amygdala and lateral ventricle. On selected test days, intracranial microinjections of MDZ was delivered into the specific brain regions of unrestrained rats. Compared to the i.p. route, central administration of MDZ in either brain area was 4-times more potent in producing increases in drug-lever responding (ED50: 8 vs. 32 µg). Drug-lever responding occasioned by intracranial MDZ injections in the lateral ventricles or basolateral amygdala was a dose-dependent manner by intraperitoneal administration of the BZ receptor antagonist, flumazenil. The antagonism by flumazenil was surmountable with higher intracranial dose of MDZ. Thus, although no site specificity was demonstrated, these data suggest the drug stimulus produced by MDZ administration was mediated via central BZ receptors. Supported by DA-04133.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
GABA-A-receptors expressed from rat α- and β-subunits in Xenopus oocytes are modulated by benzodiazepine receptor ligands.

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Institute of Physiology, University of Zurich, Switzerland; *Hoffmann-La Roche, Basel, Switzerland; **Max-Planck-Institute of Medical Research, Heidelberg, West-Germany.

The structural requirements for functionally active GABA-A-receptors were investigated in Xenopus oocytes by coexpression of clones coding for the α- and β-subunits of the GABA-A-receptor. Male Wistar rats were prepared for both binding and electrophysiological experiments. The binding of [3H]GABA was measured under voltage clamp at -70 mV, selective for chloride ions. They were inhibited by bicuculline and potentiated by pentobarbital as well as by diazepam (1-20 µM) and tatumenalin (1-10 µM). Surprisingly, the inverse agonist DCCM (3-10 µM) likewise potentiated the GABA-response. Thus, benzodiazepine binding sites are present on GABA-A-receptors expressed from rat brain α- and β-subunits. They mediate, however, only a limited spectrum of intrinsic activities. The finding is line with the biochemical localization of benzodiazepine binding sites on α-subunits of the native GABA-A-receptor.

GABA-ACTIVATED CURRENTS AND CHANNELS IN MAMMALIAN NEOCORtical NEURONS.

M. Frosch*, S. Lipton*, M. Dichter*, Department of Neurology, and Beth Israel Hospital, New York, NY 10032 (spon: L.S. Benardo).

Responses to GABA were analyzed in neocortical neurons recorded with whole cell clamp techniques. Only GABA, CI influx measurements, were performed under identical conditions. 3Ci influx measurements, addition of Mg and ATP, nor cAMP, cGMP, ATP or GTP, to the patch pipette slowed "run-down" of the GABA responses but did not affect desensitization. We also examined the effects of somatostatin-14 and 28, peptides which are thought to modulate GABA-A-receptors. Their effects are variable from cell to cell and can be positive or negative.

GABA DESENSITIZATION IN HIPPOCAMPAL NEURONS IN CULTURE.

R. Dichter and J. Prey, Department of Neurology, Graduate Hospital and the University of Pennsylvania, Philadelphia, PA 19146.

Application of GABA to hippocampal neurons in culture (WC mode with symmetric CI concentrations) via local perfusion, results in a dose-dependent desensitizing inward current (t = 6-7 sec). Desensitization may be present during ongoing synaptic activity. As examples, the continuous, long-term release with CNS goes increases the peak response to GABA and the desensitization rate while decreasing the steady state response.

We attempted to determine the rate and extent of desensitization to GABA-Areceptors could be affected by a Ca2+ or G protein-dependent process or modification of the high affinity GABA binding site. Neither intracellular perfusion with the non-hydrolysable substrate, GTPS, dramatically altered the size of the GABA response or its desensitization. Addition of Mg and ATP to the patch pipette slowed "run-down" of the GABA responses but did not affect desensitization. Application of SCN, which has been suggested to mask high affinity GABA binding sites, depressed both peak and sustained currents without affecting the rate of desensitization.

We also examined the effects of somatostatin-14 and 28 peptides which are thought to modulate GABA-A-receptors. Their effects are variable from cell to cell and can be positive or negative.

GABAergic INHIBITION IN ORGANOTYPIC HIPPOCAMPal SLICE CULTURES.

P. Stein, S. Thompson*, and Beat H. Gähwiler, Brain Research Institute, Univ. of Zürich, Zürich, Switzerland and *Dept. of Neurology, Columbus Univ., NY, 10013 (spon: L.S. Benardo).

GABAergic inhibition develops primarily after birth, has been shown to be decreased by chronic desensitization, and may decline over time in culture (McBain et al. 1988). We therefore used immunohistochemical staining with an anti-GABA antibody, to examine the GABAergic system in hippocampal slice cultures. Interneurons and terminal-like elements comprised the GABA-immunoreactivity. This effect is also opposite in direction to that observed following some chronic treatments. Thus, both forms of SOM can modulate the post synaptic GABA receptor, but their effects are variable from cell to cell and can be positive or negative.

ANATOMICAL AND PHYSIOLOGICAL PROPERTIES OF GABAergic INHIBITION IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES.

P. Stein, S. Thompson* and Beat H. Gähwiler, Brain Research Institute, University of Zurich, Zurich, Switzerland and *Department of Neurology, Columbia Univ., NYC, NY 10013 (spon: L.S. Benardo.)

GABAergic inhibition develops primarily after birth, has been shown to be decreased by chronic desensitization, and may decline over time in culture (McBain et al. 1988). We therefore used immunohistochemical staining with an anti-GABA antibody, to examine the GABAergic system in hippocampal slice cultures. Interneurons and terminal-like elements comprised the GABA-immunoreactivity. This effect is also opposite in direction to that observed following some chronic treatments. Thus, both forms of SOM can modulate the post synaptic GABA receptor, but their effects are variable from cell to cell and can be positive or negative.
398.9
DIFFERENTIAL EFFECT OF HALOPERIDOL ON GAD ACTIVITY IN DISCRETE BRAIN NUCLEI IN THE RAT. R. E. Wilcox, P. K. Randall, and R. D. Marfilez.* Institute for Neuroscience, Dep. of Pharmacol. and *Kinesiol., Univ. of Texas at Austin, Austin, TX 78712.

It has been suggested that different motor behaviors produced by dopamine (DA) agonists and antagonists may be mediated through different subtypes of DA receptors. Further, different effects may be characterized by distinct relationships with the DA receptor subtypes. If this is the case, experimental manipulations of intracellular levels should differentially affect the biochemical characteristics of the output nuclei.

We studied the effect of acute DA receptor blockade on glutamic acid decarboxylase (GAD) activity in the major subgenual output nuclei.

Male Sprague-Dawley rats (n=15) were administered the predominantly D-2 antagonist haloperidol (0.5 or 5 mg/kg, ip) or 0.01 M tartaric acid (vehicle). Micropunches were taken from the substantia nigra reticulata (SNr), globus pallidus (GP) and the entopeduncular nucleus (EP) and assayed for GAD activity using the method of Holdiness (Anal. Lett. 13: 1330, 1980). Acute challenge with 0.5 mg/kg did not alter GAD activity in the SNr by 30 and 77% in the GP and EP, respectively. The 0 mg/kg dose of haloperidol increased GAD activity by 30%.

Conclusion: brain regional GAD activity may represent a useful approach for assessing the effects of typical and atypical neuroleptics on basal ganglia efferent activity. Comparison of these results with those of nonselective or selective D-1 antagonists would be valuable in assessing the contributions of DA receptor subtypes to the function of intracellular systems.


398.10
ORTODENCY OF GABA LOCALIZATION IN PAROXYSMAL CHICK BRAIN. P. A. Levie and M. M. Beck. Dept. of Animal Sci., Univ. of Nebraska, Lincoln, NE 68583.

GABA, an inhibitory neurotransmitter and metabolic substrate in brain, has been implicated in seizure syndromes, increasing in some (Roberts et al., 1985), decreasing in others (Giesielkski et al., 1981). In 10-21d (g)g and (g) chicks, it increases (Fisman and Beck, 1986). The g syndrome includes clonic-tonic audiogenic seizures in chicks beginning at 7-10d posthatching; seizures can also be electrically elicited by 5d. In this study, brains from g and normal chicks were removed at 5, 7, and 10d and stained for GABA using immunocytochemistry in a preembedding mode) or in the presence of external Ca2+ (+78%), but not in its absence. These results indicate that asymmetrical synapses occur more frequently (61% of those analyzed) than symmetrical synapses (25%).

Asymmetrical GABAergic terminals labeled with an anti-GABA antibody and a gold probe were found in more than 85% of the total synaptic population labeled terminals are rare. The symmetrical type. Dense core vesicles are found in both types. The relatively large size of the symmetrical synapse and the presence of external Ca2+ (+78%) are characteristic features of the symmetrical synapse. The asymmetrical synapse is characterized by a large, dense core vesicle and a smaller, electron-dense, presynaptic vesicle. The asymmetrical synapse is also characterized by a large postsynaptic density and numerous, tightly packed, large round vesicles. The second type, a symmetrical synapse, lacks the thick postsynaptic density but often exhibits presynaptic immunogold probe. Thin sections were incubated with an affinity purified antibody against GABA and then labeled with a 5 nm gold-conjugated secondary antibody.

GABA and then labeled with a 5 nm gold-conjugated secondary antibody.
RESPONSE PROPERTIES OF MIDBRAIN DOPAMINERGIC AFFERENTS TO CALCIUM-BINDING PROTEIN CONTAINING BASAL
measures were utilized:

The purpose of the present experiment was to

Two regular-firing cells increased in rate but
terminate bursting activity, decreasing the probability of

In the SNC (n = 7). HAL produced a 36% increase in active DA C/T in the

where GABA degradation is inhibited with GAG. These data suggest that as midbrain DA cell number decreases there is an increase both in the number of spontaneously active cells and in the firing rates of the active cells. Research supported by NIMH (MH-30546).


There is much biochemical evidence that astrocytes are involved in neurons in the uptake and degradation of gamma-aminobutyric acid (GABA). Since most of the evidence has been obtained from astrocytic preparations of gray matter, it remains unclear whether astrocytes in white matter also take up and degrade GABA. The role astrocytes play in regulating GABA, the purpose of this study was to determine whether astrocytes in white matter contain GABA.

Adult rats were injected with gamma-aminobutyric acid (GABA)(p=0.20 mg/kg), a GABA-transaminase inhibitor, and subsequently perfused with a 1% paraformaldehyde-2% glutaraldehyde solution. Vibratome sections were cut through the brain and spinal cord and processed for preembedding light and electron microscopic GABA immunocytochemistry. Light microscopic examination revealed immunoreactive neurons but also numerous smaller immunoreactive profiles in the corpus callosum, diencephalic fiber tracts and nucleus, and spinal cord, which in the electron microscope were identified as astrocytes.

These results show that GABA is present in astrocytes located in both white and gray matter when GABA degradation is inhibited with GAG. Since astrocytes do not synthesize GABA, the GABA present in astrocytes in white matter was most likely acquired from extracellular space. Thus, the results suggest that white matter astrocytes function in a manner complementary to gray matter astrocytes by removing GABA from the extracellular space of fiber tracts.

CATECHOLAMINES II


The spontaneous discharge activity of individual monoamine-containing neurons was studied in halothane-anesthetized rats using extracellular recording techniques. Three measures were utilized: 1) mean discharge rates, 2) population characteristics of interspike interval (ISI) samples, and 3) ISI time series measures to examine patterns in the ordering of ISIs. The mean discharge rates of dopaminergic (DA) substantia nigra pars compacta and ventral tegmental area neurons and noradrenergic locus coeruleus neurons were 2.9±0.3 (n=20) and 4.5±0.2 (n=6) impulses/sec, respectively. The ISI histograms for all 3 cell populations were similar to those described by others. Additionally, the time series analysis revealed a conspicuous oscillatory tendency in the sequence of ISIs in these neurons: Consecutive ISIs tended to alternate between short and long durations, although short bursts were evident in the sequential ISIs of two of the three cell types. For all 3 cell types, long-short and short-long pairs of consecutive ISIs occurred significantly more frequently (P<0.01) than in the same samples when the order of ISIs was randomized. These oscillatory patterns are presumably produced by the operation of ionic conductances which terminate bursting activity, decreasing the probability of consecutive short ISIs, as well as conductances which serve to minimize the probability of consecutive long ISIs. The present analyses provide quantitative measures of the impact of these (and other) conductances on discharge activity.

EFFECTS OF APAMIN ON THE ELECTROPHYSIOLOGICAL RESPONSE PROPERTIES OF MIDBRAIN DOPAMINERGIC NEURONS IN VITRO. D.C. German, K. Go and A. L. Blaha. Dept. of Physiol. and Psychiat., Univ. of Texas Southwestern Med. Cntr., Dallas, TX 75235.

Midbrain dopaminergic (DA) neurons in vivo exhibit either a regular, irregular or burst firing pattern. In vitro, however, these neurons characteristically fire with a non-burst pattern. The purpose of the present study was to determine whether apamin, which blocks calcium-activated potassium channels, influences the activity of dopaminergic midbrain DA neurons. In the present study w were studied with standard Fq vitro extracellular single unit recording procedures. Cells were recorded with one microelectrode filled with a 0.1 M KCl solution containing 0.1% apamin and 0.1M dopamine (10-200uM). Apamin often caused cells to change from a ono-test to irregular or burst firing pattern (n=4). This effect is consistent with previous findings that apamin decreases the inhibitory effects of dopamine on cells firing toDA neurons possess calcium-activated potassium channels. Research supported by NIMH (MH-30546) and NIH (GM-39771).

EFFECTS OF MTPT ON THE ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF MIDBRAIN DOPAMINERGIC NEURONS IN VITRO. G.L. Bernardini, S.G. Speciale and D.C. German. Dept. of Physiol. & Psychiat. UT SW Med. Cntr., Dallas, TX 75235.

The functional properties of midbrain dopaminergic (DA) neurons were compared following lesions of several nuclei in the midbrain. The purpose of the present study was to determine whether the increase in dopamine turnover that results from cell loss is related, in part, to changes in DA neuronal impulse flow. Groups of BALB/cj mice were treated with 40, 60 and 80 mg/kg 6-OHDA-MPTP. Standard in vitro electrophysiological recording and HPLC procedures were used. The baseline firing rates of nucleus A9 neurons increased significantly (78%) as forebrain dopamine levels decreased. Autoreceptor-induced inhibition in cell firing (200 uM dopamine) was increased 40% in nucleus A10 but decreased in nucleus A9. There was also a 3 fold increase in cell incidence within nucleus A9 in the treated mice. These data suggest that as midbrain DA cell number decreases there is an increase both in the number of spontaneously active cells and in the firing rates of the active cells. Research supported by NIMH (MH-30546).

EFFECTS OF RISPERIDONE (RIS) ON SUBSTANTIA NIGRA PARS COMPACTA (SNC) DOPAMINERGIC (DA) NEURONS: COMPARISON WITH HALOPERIDOL (HAL). C.B. Davis and P.S. Blum (SPON: B.P. Damiano), Department of Biological Research, Janssen Research Foundation, Spring House, PA 19477.

The effects of acute administration of RIS, a D2, S2 antagonist on DA neurons has been described. Little is known about the chronic effects of RIS on the activity of DA neurons. In preparation for a study of chronic effects, the percent of midbrain DA neurons in the SNC that are active one hour after the injection of RIS (2 mg/kg) was compared with the percent of midbrain DA neurons that were active one hour after the injection of HAL (2 mg/kg). There were no differences in the mean discharge frequency of DA neurons in the SNC (GABAergic neurons) important in sequestering calcium-binding proteins (CBP) important in sequestering free intracellular calcium. Antibodies against these proteins were utilized to label two distinct populations of GABAergic neurons without the use of colchicine. Earlier studies (Zaborszky et al., 1986) have shown that local or intrinsic GABAergic neurons in the basal forebrain innervate cholinergic projection neurons. Since little is known about afferents to basal forebrain GABAergic neurons, we were particularly interested in identifying afferents to CBP neurons in basal forebrain areas rich in cholinergic neurons. PHA-L was iontophoresed delivered to different hypothalamic and brainstem regions and to project to the rostral forebrain, and sections processed for the simultaneous detection of PHA-L labeled fibers/terminals and CBP-containing neurons using nickel enhanced DAB/DAB double labeling technique at the EM level. CBP neurons in the lateral septal nucleus appear to receive contacts from the anterior hypothalamic nucleus, lateral hypothalamus, and locus coeruleus. PF containing neurons in the vertical and horizontal limbs of the diagonal band nuclei and lateral preoptic area are likely to be contacted by fibers originating from pontine tegmental regions and the ventral medullary reticular formation. Supported by USPHS Grant NS-23943 and 17743.

The antipsychotics RIS, an S-2, D-2 antagonist, and HAL, a selective D-2 antagonist, administered intravenously at chronic anesthetized rats while recording from midbrain dopaminergic DA neurons using standard techniques. Fifty-five DA neurons were tested for altered spontaneous activity and activity in neurons with slow (<10 spikes/sec) and rapid (>40 spikes/sec) spontaneous activity were analyzed separately. HAL increased the activity of slow neurons (n=21; 100% of initial activity at 1.27 mg/kg) but did not affect rats with rapid activity (n=11; 100% of initial activity at 1.27 mg/kg). In contrast, RIS did not affect slow neurons (n=5; 100% of initial activity at 1.27 mg/kg), but decreased the activity of rapid neurons (n=10; 85% of initial activity at 2.5 mg/kg). The spontaneous activity of 18 other RIS-treated neurons (55% of total) ceased completely with 4 HAL-treated neurons (18%). In a previous study (Soc. Neurosci. Abstr., 14:1213, 1988), RIS was unable to block inhibition of DA neurons produced by APO, but in this study, intravenous RIS and HAL reversed APO-induced inhibition of activity in 34 additional DA neurons. The mean dose of HAL to produce a 50% reversal of neural activity (ED50) was 57 µg/kg. This value was about 2.3 times less than the mean ED50 for RIS (184 µg/kg). These data show that RIS and HAL can reverse the effect of a D-2 agonist on DA neurons, but they have a different effect on spontaneous activity of DA neurons.

REPEATED ELECTROCONVULSIVE SHOCK (ECS) ALTERS SPONTANEOUS FIRING OF DOPAMINERGIC (DA) BUT NOT NORADRENERGIC (NA) NEURONES. A.J. Watson* and T.J. Svensson. Pharmacology Dept., Karolinska Institute, S-104 01, Stockholm, SWEDEN. (Sponsored by Stone.)

ECS is effective in treating various psychiatric illnesses including depression, in whose aetiology both DA and NA have been implicated. In addition, ECS has been shown biochemically and behaviourally to affect the effects of one another. We report here preliminary data on the effects of D2 agonist quinpirole and CCK octapeptide (CCK) coexist in a subpopulation of midbrain neurons and have been shown to influence the pre- and postsynaptic effects of one another. We report here preliminary data on the effects of the D2 agonist quinpirole, CCK and the CCK antagonists proglumide and CR 1409 on the firing rate of DA cells. In vitro superfusion of the femoral vein (450 µm). Extracellular recording techniques were employed. Solvent of drugs were prepared in artificial CSF and sequentially delivered via a microvalve (2 ml/min). Quinpirole (50 nM-10 µM; n=15 cells/dose) produced dose-related inhibition of the spontaneous discharge of substantia nigra zona compacta (A10) DA cells (ED50 app. 40nM). CCK (0.1-1000 nM) most affected ventral tegmental area (A10) and medial A9 cells tested (29/40). Proglumide (10 µM-100 µM; n=13) generally had no effect on A10 and medial A9 cells although a few cells were inhibited. CR 1409 (100 µM; n=12) exerted erratic effects on the firing rates of a sample population of cells but inhibitory responses predominated. Interactions among these compounds are under investigation. Supported by M442136, M441557 and Sinai Research Institute.

kappa opiate agonists have been shown to exert contrasting effects on several indices of DA cell functioning, including rewarding and aversive properties of opiates and other drugs of abuse. Mu and delta receptors mediate rewarding properties of opiates and other drugs of abuse.


The present experiments investigated the effects of the prototypical D1 antagonist SCH 23390 and the new D1 agonists NO-112 (([N-bromo-7-hydroxy-3-methyl-5-[7-benzofuranyl]-2,3,4,5-tetrahydro-1H-3-benzazepin]) on the activity of A9 and A10 DA neurons using an electrophysiological model, predictive of the clinical profile of APDs. Following 28 days (0.29 mg/kg/day) of continuous administration of NO-112 or SCH 23390 via subcutaneous mini osmotic pumps, selective decreases, 28.9% and 28.2% respectively, in the number of spontaneously active A10 DA neurons were observed. This selective effect is similar to, though smaller in degree, that reported for the atypical APD clozapine. However, in contrast to other APDs, the decrease in the number of A10 DA cells/track did not appear to result from depolarization inactivation, since the DA agonist apomorphine (10 or 50 µg/kg, i.v.) did not reverse the inhibition caused by NO-112. These results suggest that D1 DA antagonists may possess an atypical APD profile with respect to midbrain DA neurons that occurs via a mechanism other than depolarization inactivation. (Supported by MH-40832, DA-04093 and by NOVO Industri A/S.)
ALTERED RESPONSIVENESS TO HALOPERIDOL FOLLOWING MODULATION OF DOPAMINE SYSTEM RESPONSIVITY: A A. Grace. E.D. Abercrombie & M.J. Zigmond.

be attenuated by neuroleptic-induced depolarization block of DA cells, nigrostriatal "core" explants and electrochemical analysis.

PHASIC VERSUS TONIC DOPAMINE RELEASE AND THE continuum were obtained from Sprague-Dawley rats and syntheses & release) that would cause subsequent phasic DA release to occur via two processes: 1) a transient or

DA receptor stimulation, while autoreceptor antagonists facilitate stimulation, thereby averting the phasic response (see Abercrombie et al, this meeting).

supported by MH09660 & NS19608.

ALTERATIONS IN DOPAMINE CELL ACTIVITY INDUCED BY PHASIC VERSUS TONIC Dopamine RELEASE AND THE MODULATION OF DOPAMINE SYSTEM RESPONSIVITY: A HYPOTHESIS FOR THE ETIOLOGY OF SCHIZOPHRENIA. A.A. Grace, E.D. Abercrombie & M.A. Sigmon. Dept. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The dopamine hypothesis of schizophrenia, in its simplest form, suggests that this disorder is caused by DA system hyperactivity. However, there is evidence from experimental findings that are inconsistent with this model: 1) increased DA metabolites have not been observed in the striatum of DA depleted rats, i.e., a pacemaker-like firing was seen. In contrast, A10 cell firing was unaltered. Ritalin blocked the effect of PFC inactivation on all A10 neurons (10/10), while amphetamine blocked the chronic (negative, type II) schizophrenia, a disorder characterized by hyperactivity and poor response to traditional neuroleptic treatment.

Pharmacology, Univ. of Colorado. Denver, CO 80262.

STIMULUS FREQUENCY-DEPENDENT DOPAMINE AUTORECEPTOR ACTIVITY was observed in both cases by exposure to stressors or acute treatment with neuroleptics. In this study we examined the effects of haloperidol (HAL) on the activity of nigral DA neurons and motor behavior 4-6 weeks post-lesion (>85% depletion of nigrostriatal tract increased from .04+.08 to ,13 ± .13  (n= 6

Behavioral studies showed that the same dose of HAL (1p) could induce a profound akinetik and cataleptic state (as measured by open field and raised platform tests) in the DA depleted animals while having no effect on controls in these tests. Studies are underway to determine the time course for the development of this alteration in the behavioral and electrophysiological response to HAL. Supported by MH09606 & NS19608.


Clinical neurophysiological studies have demonstrated that in patients with Parkinson's disease, a relatively consistent feature of chronic schizophrenia with negative symptoms is the possibility of a state of hypoactivity on the mesolimbocortical dopamine (DA) system, which have recorded the spontaneous activity of A10 DA neurons in animals with PFC during PFC inactivation induced by application of dry ice to the skull overlying the PFC. Application of dry ice to the skull overlying the PFC, PFC cooling regularized the firing and abolished brisk firing of A10 cells, i.e. a pacemaker-like firing was seen. In contrast, A10 cell firing was unaltered. Ritalin blocked the effect of PFC inactivation on all A10 neurons (10/10), while amphetamine blocked the chronic (negative, type II) schizophrenia, a disorder characterized by hyperactivity and poor response to traditional neuroleptic treatment.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
TIME COURSE OF THE EFFECTS OF KAINTETE-INDUCED PREDNUCLEOPHONINE TEGMENTAL NUCLEUS (PPN) LESION SUGGESTED DEVELOPMENT OF CIRCADIAN RHYTHM NIGRA-PARSCAPTA COMPACTA DOPAMINE CELL DEPOLARIZATION INACTIVATION RESULT FROM LOSS OF PPN FUNCTION. M. Beninato, R.P. Pan and P. Jacobson. NINDS, Bethesda, MD 20892.

Previous studies (1) have shown a significant decrease in the number of spontaneously active (SA) substantia nigra pars compacta (SNpc) neurons that occurred 7 days following kainite lesion of the PPN. Current studies using amipomine pretreatment and GABA antagonist treatment indicate that the SNpc neurons appear to be in a state of depolarization inactivation. Using previously described population study techniques (1) in chronically hydrated anesthetized rats, the time course of the development of this effect was studied. At 1 hour post lesion, the number of SA DA cells/pass (0.32±0.04, n=4) was significantly reduced from controls (0.82±0.09, n=6). Apomorphine pretreatment (80µg/kg) reversed this effect (0.92±0.13 cells/pass, n=6). This indicated that the silent DA neurons are in a state of depolarization inactivation, resembling the condition seen at 7 days (0.5±0.07 cells/pass, n=9). At 12 hours post lesion, however, the number of SA DA cells/pass rebounded to control levels (0.58±0.67, n=6). These results suggest the depolarization inactivation observed at 1 hour and 7 days are due to different mechanisms. While the depolarization seen at 1 hour may be the result of an initial kainite-induced excitation of the PPN, this effect on the SNpc DA neurons is apparently not sustained. The depolarization inactivation observed at 7 days may be due instead to the destruction of the PPN and subsequent alterations of neuronal influences on the SNpc DA system. L. Beninato et al, Soc. Neurosci. Abstr., 14(1): 407, 1988.

THE EFFECT OF CHRONIC NICOTINE ON THE ELECTROPHYSIOLOGICAL RESPONSE OF A9 AND A10 NEURONS TO SYSTEMIC NICOTINE. K.-W. Yung, W.A. Curran, and T.C. Heathall (SPON: K. Smith). Dept. of Pharmacology and Neurosurgery, St. Louis Univ. Sch. of Medicine, St. Louis, MO 63104.

We have previously observed that the systemic administration of nicotine (NIC) increases the firing rate of midbrain dopamine (DA) neurons (Eur. J. Pharmacol. 141:395, 1987). In the present studies, the effect of i.v. NIC on the firing rates of A9 and A10 DA neurons was studied by extracellular single unit recordings. I.v. NIC increased the firing rate of both A9 and A10 cells in naive rats and as previously observed by us, NIC was more potent and efficacious in activating A10 than A9 neurons. In contrast, chronic treatment with NIC for 7 or 14 days did not alter the increase in the firing rate of i.v. NIC in A9 cells. In contrast, chronic treatment with NIC for 7 or 14 days did not alter the increase in the firing rate of i.v. NIC in A9 cells. 4 days. It is concluded that systemic NIC preferentially stimulates A10 compared to A9 cells and this difference is augmented further by chronic NIC exposure. (Supported by DA 02688.)

SECOND MESSAGERS: CALCIUM

400.1

BIPHASIC MUSCARINIC-INDUCED CHANGES IN INTRACELLULAR CALCIUM OF SUSPENDED SINGLE HUMAN ASTROCYTOMA CELLS. S.A. Oglesby and B.S. Pallotta. Curriculum in Neurobiology and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599.

Using the intracellular fluorescent calcium-binding dye Indo-1 in conjunction with a fluorescence activated cell sorter, stimulation of 1321N1 human astrocytoma cells by muscarinic agonists was shown to result in a biphasic increase in the concentration of intracellular calcium of suspended cells. At least one component of this response is dependent on the presence of extracellular calcium. This biphasic calcium mobilization response in these cells suggests the involvement of at least three components which might be tenatively described as: a transient intracellular calcium release and involvement of at least three components which might be tenatively described as: a transient intracellular calcium release and a more prolonged entry of extracellular calcium, and an efflux or further resequestration of cytosolic calcium.

400.2


Rat nodose ganglion neurons have 3 calcium current components similar to T, L and N currents in other sensory neurons. Neuropeptide Y selectively reduced the N current component via a pertussis toxin (PTX)-sensitive mechanism (Soc. Neurosci. Abstr. 14(1):465, 1988), suggesting that an inhibitory GTP binding (G) protein (G/Y) couples certain receptors to voltage-gated calcium channels. We examined the effects of 3 calcium current activators via a G-protein sensitive (stable their derivatives GDP-β-S (inactive ligand) and GTP-γ-S (activates G proteins) on the 3 calcium current components of rat nodose ganglion neurons. Currents were recorded from acutely-dissociated neurons from 7-10 d rats using the whole-cell variation of the patch clamp technique. The bath medium contained (mM): 10 HEPES, 67 choline, 100 TEA, 5.6 glucose, 5.3 KCl, 0.8 MgCl₂, 5 CaCl₂ (pH 7.4). The recording pipette medium contained 10 HEPES, 140 CsCl, 10 EGTA, 5 ATPMg and either 0.1 GTP, GDP-β-S, or GTP-γ-S. In control neurons (GTP) and N and L current components decreased during the 20 min recording period and GTP-β-S did not affect the amplitude, time to peak current, rate of inactivation, voltage ranges of activation or inactivation, or rate of "run-down" of calcium current components compared to controls. In contrast, GTP-γ-S differentially reduced the magnitude of calcium current components N>T>L and increased the time to peak of N currents 3-4 fold. Pretreatment with PTX reversed the effects of GTP-γ-S. Thus, GTP-γ-S differentially affected the calcium current components in nodose ganglion neurons via a G-protein of the G/Y type. Supported by VA Research Award to JWW, NIH 01019 to RAG, DA05345 to TR-J and DA04122 to RLM.

400.3

PROTEIN KINASE A ENHANCES, AND ATP-γ-S REDUCES CALCIUM CURRENT COMPONENTS OF RAT NODOSE GANGLION NEURONS. R.A. Gross, M.D. Uhler* and R.L. Macdonald. Dept. of Neurology and #Internal Medicine, U of Michigan, Ann Arbor, MI 48104.

Acutely-dissociated rat nodose ganglion neurons have 3 calcium current components, similar to T, L and N currents of other sensory neurons. We used the purified catalytic subunit of protein kinase A (PKA) or the ATP analog ATP-γ-S, included in the recording pipette, to determine if kinase-mediated phosphorylation affected calcium currents in these neurons. In other preparations, cyclic AMP or PKA increases or removes inactivation of N and L current components. The effect of ATP-γ-S on calcium currents from acutely-dissociated nodose ganglion neurons was studied. A transient calcium release and an influx of extracellular calcium, and an efflux or further resequestration of cytosolic calcium. We used the whole cell variation of the patch clamp technique to record calcium currents from acutely-dissociated rat nodose ganglion neurons from 7-10 d rats. The bath medium contained (mM): 10 HEPES, 67 choline, 100 TEA, 5.6 glucose, 5.3 KCl, 0.8 MgCl₂, 5 CaCl₂ (pH 7.4). The recording pipette medium contained 10 HEPES, 140 CsCl, 10 EGTA, 5 ATPMg and either 0.1 GTP, GDP-β-S, or GTP-γ-S. In control neurons (GTP) and N and L current components decreased during the 20 min recording period and GTP-β-S did not affect the amplitude, time to peak current, rate of inactivation, voltage ranges of activation or inactivation, or rate of "run-down" of calcium current components compared to controls. In contrast, GTP-γ-S differentially reduced the magnitude of calcium current components N>T>L and increased the time to peak of N currents 3-4 fold. Pretreatment with PTX reversed the effects of GTP-γ-S. Thus, GTP-γ-S differentially affected the calcium current components in nodose ganglion neurons via a G-protein of the G/Y type. Supported by VA Research Award to JWW, NIH 01019 to RAG, DA05345 to TR-J and DA04122 to RLM.

400.4


The Ca²⁺-sensitive fluorescent dye, Fura-2, has been utilized to investigate regulation of internal [Ca²⁺] in synaptosomes from specific regions of rat CNS including striatum. In striatal synaptosomes first equilibrated at 37° for 60 min apd then loaded with Fura-2 acetoxymethyl ester, basal [Ca²⁺] was stable at about 115 nM; following depolarization induced by 50m M K⁺ within 2 seconds [Ca²⁺] increased to a new level of about 370 nM. Addition of 1µM 1-15-[isopropylamino]-2-phenylpiperazine (W7), an inhibitor of protein kinase C, caused small but significant decrease (~20%) in basal [Ca²⁺] and a marked attenuation (~80%) of the effect of depolarization. The calcium inhibitor, N-(6-aminohexyl)-5-chloro-1-glycylglycine (CMZ) at 10µM depressed basal [Ca²⁺] slightly and almost completely abated the K⁺-induced increase in [Ca²⁺]. The calcium antagonist, dihydrolazolium (DNZ), at 10µM, unexpectedly increased [Ca²⁺] to several-fold basal level, probably due to a calmodulin-independent effect. Correspondingly, CMZ, but not W7, caused release of transmitter (e.g. -amino butyric acid and serotonin), H7 antagonized transmitter release. W7, H7 or CMZ did not alter striatal synaptosomal cyclic AMP. The effects of H7 and W7 support roles for calmodulin and protein kinase C in depolarization-induced [Ca²⁺] influx and release of internal [Ca²⁺].

cDNA clones encoding human (H) muscarinic acetylcholine receptor (mChR) subtypes HM1, HM2, HM3 and HM4 were introduced by the calcium phosphate method into Chinese hamster ovary (CHO) cells lacking endogenous mChRs. Subsequently transfected clonal cell lines were isolated and characterized previously (Peralta et al. 1987). Single cell Ca** measurements were recorded using the Ca**-r dye indicators FLO-3 AM (on a confocal microscope) and INDO-1 AM (on a flow cytometer). Acetylcholine (ACh)-induced increases in peak Ca** for flow cytometry are summarized in terms of fluorescence change by digital imaging of single PC12 cells (Clapham, D.E., et al., BBRC, 159:976, 1989). These data confirm the dual mechanisms of BK actions on BK**-regulated Ca** responses of HM1 and HM4 were the largest, the most sensitive to ACh and the least affected by PTX treatment (100ng/ml, 14-20 hrs). In contrast, the HM2 and HM3 responses were smaller and very sensitive to PTX. Chimeric mChRs, stably transfected into CHO cells, are currently being examined. This work was supported by NIH (D.E.C.) and Genentech (E.G.P.).

CONTROL

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The Ca** responses of HM1 and HM4 were the largest, the most sensitive to ACh and the least affected by PTX treatment (100ng/ml, 14-20 hrs). In contrast, the HM2 and HM3 responses were smaller and very sensitive to PTX. Chimeric mChRs, stably transfected into CHO cells, are currently being examined. This work was supported by NIH (D.E.C.) and Genentech (E.G.P.).

NEUROTTRANSMITTER STIMULATED INCREASES IN MASS LEVELS OF INS (1,4,5)P3 IN BRAIN SLICES AS MEASURED BY IP3 RADIORECEPTOR ASSAY. D.S. Bredt, S.H. Snyder, Dep. of Pharmacology and Molecular Sciences and Department of Neuroscience Johns Hopkins Univ. Sch. of Med. Balt., MD 21205.

The IP3 receptor from crude rat cerebellar membranes was used to create a simple, sensitive, and specific radioreceptor assay that allows the determination of mass levels of IP3 in biological tissues (Bredt, D.S., et al., BBRC, 159:976, 1989). The IP3 receptor's specificity for IP3 is less than 1mole of IP3, and it is specific for Ins(1,4,5)P3 as demonstrated by both enzymatic and chromatographic studies. Using this assay we detect substantial increases in mass levels of IP3 in vitro slices of rat cerebellum in response to excitatory amino acids (EAA). Using 500 uM quisqualate, mass levels of IP3 increase to 350% of baseline within 10 sec., decreasing to only 150% by 1 sec. The rank order of EAA's in terms of potency and efficacy is quisqualate > glutamate > kainate. These data demonstrate, for the first time, that neurotransmitters can stimulate large, transient increases in mass levels of IP3 in brain slices.

1989, in press) appears to act as an intracellular Ca** channel. Two distinct mechanisms of Ca** regulation have been demonstrated to function in this system. The first is a membrane-bound protein (calmodulin) distinct from the IP3 receptor, which mediates an ETA reversible inhibition of binding with a Km for Ca** of 300nM (Danoff, S. et al., Biochem. J., 254:701, 1988) This protein has been further purified and characterized. A second mechanism is mediated by a membrane-bound, Ca**-dependent IP3-phosphatase. This protein has been partially purified and characterized. The phosphatase has a Km for IP3 of ~10uM and an absolute dependence on Mg**.


InsP6 stimulates **Ca** uptake in cultured cerebral neurons and anterior pituitary cells. The influx of extracellular **Ca** is accompanied by translocation of protein kinase C and Km for cerebellar neurons, by excitatory amino acid release. The action of InsP6 is reproduced by inositilpentakisphosphate, but not by inositol-1,3,4,5-tetrakisphosphate (InsP4). Low concentrations (5-100 uM) of divalent cations (Ca**, Mg**, Ni**). **Ca** influx by InsP6. We have studied [F]InsP6 binding in crude synaptic membranes from cerebral homogenates of rat and monkey neurons, incubated at 37°C in Tris-HCl (PH 7.4). **InsP6 binds to a single population of specific and saturable recognition sites with a KD in the low nanomolar range. [F]InsP6 binding is temperature-dependent, being virtually absent at 0°C. Specifically the KD is displaced by InsP6 and InsP4 but not by glucocereb. We speculate that InsP6 stimulates a specific class of membrane recognition sites, triggering the influx of extracellular **Ca** in target cells.
Prolonged Prohormone Ester PreTreatment Desensitizes the X Opiate-Induced Inhibition of Ca^2+ Influx in Rat Spinal Cord-Derived Neuroblastoma Cells. T. Vogel, Attali*, D. Sayegh and S.-Y. Nahm*. Dept. of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel.

Ca^2+ influx into rat spinal cord-derived neuroblastoma cells is stimulated by X opioids. Opiate agonists activate the K^+ channel (UG-08468, tetrodotoxin, ethylketocyclacocine, dynorphin) profoundly depresses the K/Bay k-stimulated Ca^2+ uptake (Attali et al., JBC, 264:347, 1999). Additional activation of the noradrenergic X ester TPX (activator of protein kinase C) to the Ca^2+ influx assay (5 min) decreased the K/Bay k-stimulated Ca^2+ uptake. PDDA inhibition at 1 µM, but did not affect the inhibition produced by the opiates. However, prolonged preincubation (24 h) of the cells with 100 nM TPX abolished the capacity of the X opiates, as well as of TPX itself, to inhibit the 45Ca^2+ influx through the voltage-dependent Ca^2+ channels. A significant desensitization of the opiate effect was already observed following 1 h pretreatment with 100 nM TPX. This desensitization by TPX of the opiate inhibitory effect was dwarfed-dependent with Cd^2+ in the nanomolar range. The inotropic phenothiazine (4-Phenothiazine-13-acetate) did not affect the opiate inhibition. These results suggest that protein kinase C affects Ca^2+ channel activity and has a role in the modulation of Ca^2+ channel activity by X opiates.

BRADYKININ MODULATES POTASSIUM AND CALCIUM CURRENTS IN RAT PHEOCHROMOCYTOMA CELLS A. Villarroya*, N. Marmas* and P.R. Adams*, Department of Nutritional Sciences, SUNY SB, Sunny Brook, NY 11794

PC12 cells with 50% polyethylene glycol and exposed to 50 ng/ml NGF for 2-8 days have been voltage-clamped in the whole-cell mode. The effects of bath-applied bradykinin (BDK) (1 µM) on ionic conductances were studied. BDK produced a membrane depolarization due to the development of an outward current (Re) that was blocked with 200 µM D-600. The development of Ins(1,4,5)P3 was accompanied by a two to threefold increase in Ca^2+, and was prevented by 100 µM BAPTA and was the pipette (cf. Paoletti et al., Biochem. 263:17350, 1988). The hyperpolarization was followed by a desensitization attributable to inhibition of an M current (I). In contrast to NGF-15 cells (of R. H. and Brown D.A., Nature 323:169, 1986), in the presence of 10 µM bath-applied 1 µM BDNF, 12,13-Dibromo-PDBU, 250 µM L-α-Disodium, 250 nM of 20 µM Ac-20 4’,5’-OAc-Glycerol (KD IP3) in the electrode produced a reduction of Ins(1,4,5)P3 (up to 100 µM) or 10 µM BAPTA did not affect BDK-mediated inhibition of Na when included in the recording pipette. These challenges with 10 µM caffeine, no effect on K or a negligible increase in Ca^2+ was detected. Calcium currents were recorded in K^+ and with CaCl and 1 mM BAPTA in the electrode. Calcium was increased up to 200 or 400 ms and maintained at the level of 1 µM for 200 or 400 ms. Under these conditions, BDK produced a reversible reduction of the calcium currents, without affecting significantly its decline during the pulse. In some cells a reduction of the holding current was observed. BDK’s action on the voltage-dependent calcium currents may be mediated by intracellular calcium, because it was suppressed by inclusion of 10 mM BAPTA in the pipette. PDBu (1 µM) produced a small reduction of the current measured after 400 ms. This effect took several minutes to develop and was partially reversible.

SECOND MESSAGES: PHOSPHOinosITIDE TURNOVER

INCREASED 32P2 INCORPORATION INTO BRAIN PHOSPHOinosITIDES IN HEREDITARY MODEL OF EPILEPSY J.M. Tochuk*, R.L. Wodzicki*, D.D. Johnson and E.C. Fedder*. Dept. of Pharmacology, College of Medicine, Univ. of South Carolina, School of Medicine, Charleston, SC 29425.

Epileptic fowl is a consequence of a spontaneous recessive mutation which is characterized by a low seizure threshold. The rate of 32P2 incorporation into phosphatidylinositol (Pi) phosphatidylinositol-4-phosphate (PiP) and phosphatidylinositol-4,5-phosphate (PiPP) was studied in synaptosomes prepared from the forebrains of carrier and epileptic birds. Synaptosomes were isolated in a standard fashion and resuspended in a buffer containing 1.10 mM NaCl, 5 mM KCl, 1.3 mM MgCl2, 1.3 mM CaCl2, 1 mM phenoxybenzamine, 10 mM glucose and 50 mM imidazole pH 7.4. An aliquot (1 mg/L) protein of the synaptosomes was incubated at 37°C with 5 µCi of 32P-iTPC (IP) and buffer in a total reaction volume of 500 µl for periods up to 50 min. Labelling was terminated by the addition of a pre-mixed solution of CHCl3/MeOH:2H2O:4 N HCl (2:10:2:9). The aqueous layer was washed with CHCl3 and the pooled pHCl extracts were evaporated to dryness under N2. The dried extract was resuspended in CHCl3/MeOH (2:1) and the lipids were separated on silica gel 60 plates with CHCl3/Methanol/CH3COOH/H2O (80/20/24/16). 32P- PiP, Pi and phosphatidic acid (PA) were identified with iodine vapor using appropriate standards. The lipid spots were scraped and the radioactivity was determined with an amount of 32P incorporated into the phosphoinositide lipids and PA was approximately 35% higher in synaptosomes prepared from epileptic than in the carrier birds. These data indicate that a de novo phosphoinositide synthesis is increased in the brain of the epileptic fowl as compared to the carrier birds. Supported by the MRC.

LOW LEVELS OF INOSITOL PHOSPHATE FORMATION ARE ASSOCIATED WITH THE OPENING OF DIHYDROPYRIDINE-SENSITIVE Ca^2+ CHANNELS BUT NOT Ca^2+ MOBILIZATION IN BOVINE ADRENAL CHROMAFFIN CELLS. K.A. Stauderman, R.M. Fruss* and N.M. Moravsky*. Merrell Dow Research Institute, 2110 E. Gaither Rd, Camden, TN 38321.

In single bovine adrenal chromaffin cells loaded with fura-2, histamine concentrations from 1-30 nM elicited slow, oscillatory increases of intracellular calcium (1Ca^2+). These responses were K^+ mediated, and were apparently due to Ca^2+ channels which they were antagonized either by 0.1 µM magnesium, 20 µM gadolinium (Gd^3+), or by 1 µM (1-1000-19, a dihydropyridine antagonist. In cell cultures, 3-30 nM histamine stimulated the accumulation of inositol 4- and 1-monophosphate (Ins(4)P and Ins(1)P), with Ins(1)P predominating. Concentrations > 100 nM histamine produced a rapid and largely transient release of Ca^2+ (Ca^2+), often followed by a second transient of variable size, which then declined to a new plateau above prestimulation levels. Removal of external Ca^2+, 20 µM Gd^3+, or 1 µM (1-1000-19 did not affect the initial transient, supporting a role for Ca^2+ mobilization in this response. Interestingly, ≥ 100 nM histamine stimulated more Ins(4)P accumulation than Ins(1)P.

These data suggest that conditions causing preferential accumulation between Ins(1)P and Ins(4)P may represent the coupling of the [Ca^2+]i flux to enzyme activation.

MUSCARINIC RECEPTOR-MEDIATED INCREASE IN CALMODULIN ACTIVITY IN SK-N-SH HUMAN NEURONOBLASTOMA CELLS. L.A. Mangela* and M.E. Greaves, Department of Pharmacology, The University of Michigan, Ann Arbor, MI 48109.

The enzyme systems and processes mediated by the Ca^2+ binding protein, calmodulin (CaM), are thought to play an important role in stimulus-induced Ca^2+ signalling. The effects of a muscarinic receptor-mediated Ca^2+ flux on the activity and distribution of CaM was examined. We found that exposure of SK-N-SH cells to the muscarinic agonist carbacol results in a concentration and time-dependent increase in CaM activity in the cytosol. The EC50 for the response to carbacol is 1-12 µM, which correlates with the EC50 of carbacol for the phosphoinositide response of 10-30 µM, and the EC50 of 8 µM of the agonist oxotremorine-M for peak rise in intracellular external Ca^2+ (Ca^2++). The maximal increase in cytoplasmic CaM with 10 µM carbacol from a control value of 2.0 ± 0.6 ng CaM/µg protein to 9.2 ± 2 ng CaM/µg protein. The increase in CaM activity for 100 µM carbacol occurred by 5 minutes and appeared to last for at least 30 minutes, with the maximal increase in the cytosol from 3.9 ± 0.3 ng CaM/µg protein to 10.7 ± 2 ng CaM/µg protein occurring at 15 minutes. There was no difference between the control cytosolic CaM levels and those measured after 2 hours of incubation with carbacol, and no difference in the CaM activity in the membrane fractions. The response to 10 µM carbacol was completely blocked by 1 µM atropine, and the response did not occur when the cells were stimulated with the nicotinic agonist diethylenetriamine piperazine. These results suggest that the muscarinic receptor-mediated rise in CaM in the cytosol of SK-N-SH cells. This increase in cytosolic CaM activity may represent the coupling of the Ca^2+ flux to enzyme activation.
In all three regions studied, PCP significantly inhibited the release of 
Medic in e, St. Louis, MO 63104.

PCP has been shown to have agonist properties at sigma opiate receptors, its actions were compared to those of (+)SKF 10,047, a sigma agonist. (+)SKF 10,047 inhibited the PCP-sensitive component of phosphoinositol (PI) hydrolysis with IC50s of 34, 53 and 230 µM, respectively. Haloperidol and 3-(tolyproxyphenyl)-N-(1-propyl)-piperidine (3-PPP) caused a maximal 15-40% inhibition at concentrations of (+) pentazocine 34, 53 and 230 µM, respectively. These results suggest that the increase in PCP activity induced by GLUT is not mediated by a known EAA receptor in the neonatal rat spinal cord. It is not clear why NMDA receptor antagonists do not reduce at least a proportion of the GLUT response.

These results suggest that the increase in PCP activity induced by GLUT is not mediated by a known EAA receptor in the neonatal rat spinal cord. It is not clear why NMDA receptor antagonists do not reduce at least a proportion of the GLUT response.

401.5 EFFECTS OF SIGMA COMPOUNDS ON AGONIST-STIMULATED PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN. S.M. Candara*, T. Coccini*, L. Manzoni and L.G. Costa (SPON: J. Franck). Dept. of Pharmacology, Univ. of Pavia Medical School, Pavia, Italy.

Sigma receptors are non-opioid, non-dopaminergic receptors which are implicated in psychosis and in antipsychotic drug efficacy. The second messenger system coupled to these receptors is still unknown. Recently, an inhibitory effect of various sigma compounds on carbachol-stimulated phosphoinositide (PI) metabolism has been reported (Eur. J. Pharmacol. 140, 399, 1988). We have investigated the effect of sigma compounds on carbachol-stimulated PI metabolism in rat cerebral cortex slices. (+) Pentazocine, 1,3-di-o-tolyglycine (DTG) and N-allylnormetazocine (SKF-10,047) inhibited carbachol-stimulated PI hydrolysis with IC50s of 34, 53 and 230 µM, respectively. Haloperidol and 3-(hydroxyphenyl)-N-(1-propyl)-piperidine (3-PPP) caused a maximal 15-40% inhibition at concentrations of (+) pentazocine 34, 53 and 230 µM, respectively. Haloperidol and 3-(hydroxyphenyl)-N-(1-propyl)-piperidine (3-PPP) caused a maximal 15-40% inhibition at concentrations of (+) pentazocine 34, 53 and 230 µM, respectively.

401.4 EFFECTS OF DOPAMINE AND CHOLECYSTOKININ ON PHOSPHATIDYL-INOSITOL-TURNER GANTRY - Ca2+ SIGNALLING SYSTEM IN THE RAT STRIATUM. D. Tanaka, T. Tate, R. Kuhlman and T. Mitsuno*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

Dopamine plays an important role on psychomotor functions in the central nervous system. In particular, the striatum contains the highest density of dopaminergic terminals from the substantia nigra within the brain. On the other hand, cholecystokinin (CCK) and its receptors richly exist in the striatum and modulate functional relationship between dopamine and CCK neurons has been demonstrated. In the present study, the authors studied effects of dopamine and CCK on phosphatidylinositol (PI) turnover and intracellular Ca concentration ([Ca2+]i) in the rat striatum. PI turnover was measured according to a ferric chloride method using striatal slices. The level of [Ca2+]i was measured by fura-2 fluorometry in cultured striatal neurons on a single cell basis. It has been demonstrated that dopamine D2 receptors in the pituitary inhibit PI turnover. In our experiments, dopamine stimulated PI turnover dose-dependently in the striatum. CCK had no effect on PI turnover, while the 5-HT2 receptor increased [Ca2+]i. It is noteworthy that these novel actions of dopamine and CCK on the second messenger system were found in the striatum.

401.6 EXCITATORY AND SULFUR AMINO ACIDS MODULATE BRAIN PI HYDROLYSIS. X. Li and R.S. Jope. Dept. of Pharmacology, Univ. of Alabama, Birmingham, AL 35294.

We reported earlier that glutamate inhibited NE-stimulated PI hydrolysis in rat brain slices by inhibiting phospholipid synthesis through a quisqualate-selective site. We further found that sulfur-containing amino acids also inhibited NE-stimulated PI hydrolysis in rat brain slices. Of the tested sulfur-containing amino acids, L-cysteine was the most potent, inhibiting the NE-induced response by 42% and 85% at concentration of 50 µM and 500 µM, respectively. L-homocysteate slightly potentiated PI hydrolysis at a concentration of 100 µM, but it was inhibitory at 500 µM. The thios-forms of cysteine and homocysteate were much less potent than were the L-isomers. L-cysteine, L-cysteine sulfinate and d-cysteine-sulfinate also inhibited PI hydrolysis, but the effect was moderate or mild compared to L-cysteine. Sulfur-containing amino acids did not cause cell lysis or affect the integrity of the PI second messenger system.

401.7 PCP INHIBITS CARBACHOL-INDUCED PHOSPHOINOSITOL HYDROLYSIS IN THE RAT BRAIN. J.M. Brog and M.G. Beinfeld. Department of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63128.

We have previously reported that while phorbol esters increased CCK release in various regions of the rat brain, phencyclidine had an inhibitory effect. In order to examine a possible relationship between inhibition of CCK release and the phosphoinositide cycle, we examined the effect of PCP on carbachol-induced phosphoinositol hydrolysis in rat brain slices from cortex, caudate-putamen, and the hippocampus and cerebellum. In all three regions studied, PCP significantly inhibited the carbachol-induced 1H-myoinositol-1-phosphate accumulation, exhibiting the greatest potency in the cortex at 0.1 µM. Since PCP has been shown to have agonistic properties at sigma opiate receptors, its actions were compared to those of (+)SKF 10,047, a sigma agonist. (+)SKF 10,047 inhibited the carbachol-induced phosphoinositol hydrolysis in similar concentration ranges as those reported for excitatory amino acids. It is also possible that PCP is at least partially acting through the sigma opiate receptor to inhibit the carbachol effect. However, further studies will be required to elucidate its mechanism of action, and how this action of PCP may be related to its effect on CCK release. Supported by NIH NS38667.

401.8 ATP INDUCES POLYPHOSPHOINOSITIDE BREAKDOWN AND AN INCREASE IN CYTOSOLIC FREE CALCIUM LEVELS IN NEUROBLASTOMA X GLIOMA HYBRID CELLS. T.A. Litw, K.D. Lustig*, M.G. Sportofi*, G.Y. Sun and G.A. Weiskant*. Department of Biochemistry and Sinclair Research Farm, University of Missouri-Columbia, Columbia, MO 65201.

Extracellular ATP exhibits hormonal-like properties in the peripheral and central nervous systems. In this study, the addition of 2 µM ATP to a neuroblastoma X glioma hybrid cell line (NG108-15) was found to induce a transient increase in the level of cytosolic free calcium ([Ca2+]i). The increase in [Ca2+]i was observed within 10 s after addition of ATP and then declined to the initial level within one minute. However, the ATP-induced increase in the [Ca2+]i was greater in the presence than in the absence of extracellular calcium. Increases in the [Ca2+]i correlated with the concentration of the fully ionized form of ATP (ATPa) in the medium. Other nucleoside 5'-triphosphates, including UTP, ITP, TTP and GTP also induced an increase in the [Ca2+]i. The addition of ATP to cells pre-loaded with [32P]inositol resulted in a rapid increase in the level of phosphatidylinositol 4,5-bisphosphate (PIP), the lipid precursor of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DG). ATP also caused an increase in the level of [32P]labeled phosphatidic acid, presumably as a result of phosphorylation of DG. These findings suggest that extracellular ATP activates a signal transduction pathway in NG108-15 cells involving the formation of IP3 and the mobilization of Ca2+.

We have been using the NG108-15 clonal cell line as a model system to study the regulation of phosphoinositide (PI) hydrolysis. NG108-15 cells were grown in MEM with HAT and 10% FBS and labeled for 2 days with [3H]-inositol. The media was removed and the cells pre-incubated for 15 min in Ringert's LiCl. Bradykinin (BK) was then added for various times and the reactivity decreased with time. Inositol phosphates were separated by ion-exchange chromatography. BK stimulated PI hydrolysis in a dose-responsive fashion reaching a maximum at a concentration of 10-25 nM. The response was extremely rapid; an increase in IP_3 was detectable at 5 sec and peaked at 15-30 sec at approximately 300% of basal values. Stimulation then gradually declined returning almost to baseline by 2 min. IP_2 accumulation followed a similar pattern after a slight time delay. Although basal values of both IP_3 and IP_2 were higher than either IP_1 or IP_4, minimal stimulation in response to BK was seen over this time period. Low (1-10 M) concentrations of LiCl appeared to have subtle effects on the kinetics of IP_3 production/metabolism in response to BK, slightly delaying the time point of peak accumulation. Thus at earlier times, Li appeared to inhibit IP_3 while after longer stimulation, the response was equal or slightly potentiated.


Angiotensin II (AII) increases tyrosine hydroxylase and proenkephalin A mRNA levels in cultured bovine adrenergic (BAM) cells. The increases are inhibited by spingosine, suggesting the involvement of protein kinase C (PKC). However, a 30 min incubation with AII, which activates PKC, is not sufficient to change the mRNA levels. Therefore, we examined whether long-term incubation with AII affects PKC. AII (200 nM) increased the particulate PKC activity by 400% after 6 and 18 hrs, respectively. Similar changes were produced by 2 mM AII at 12 hrs. Total (soluble + particulate) PKC activity was increased 1.5-fold by 18 hrs. To determine the time required to induce changes in mRNA levels, cells were incubated with AII (20 nM) for 24 hrs and at 2, 6, 12, and 18 hrs. Twelve, but not 3 or 6, hr stimulation of AII receptors was sufficient to increase the mRNA levels. Thus, in addition to a short-term activation, AII induces a long-term increase in PKC activity. The long-term activation may mediate the effects of AII on the gene expression.

LOSS OF MUSCARINIC RECEPTORS AND OF QUININE-NUCLEOTIDE-STIMULATED PPI HYDROLYSIS IN HUMAN NEOBLASTOMA CELLS UPON LONG-TERM TREATMENT WITH TPA OR MEZERENZ. C.G. Cowiff and S.K. Fisher. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48104.

The actions of tumor promoters on the coupling of muscarinic receptors to hydrolysis of phosphatidyl inositol (PI) in NG108-15 cells was investigated in human neuroblastoma cells. 50 nM 12-0-tetradecanoylphorbol (TPA) to these cells in digitonin-permeabilized cells. These results suggest that TPA and mezerein decrease PPI hydrolysis by both a reduction in muscarinic receptor number and inhibition of guanine-nucleotide-stimulated PPI turnover. (Supported by NIMH Grant MH42652 and NIMH Training Grant MH57941).

HOMOLOGOUS DESENSITISATION OF BRADYKININ-INDUCED INOSITOL POLYPHOSPHATE FORMATION IN NEO-NATAL RAT DORSAL ROOT GANGLION (DRG) NEURONES. G. Burgess* and M. McNeill*. (Spon. E.A. Grove). Sandoz Institute for Medical Research, 5, Gower Place London WC1E 6BN, U.K.

Bradynadin (BK) induces inositol phosphate (IP) formation in cultured dorsal root ganglion (DRG) neurones. Serotonin also increased IP_3 formation in these cultures. The responses to maximally effective concentrations of the two agonists were additive, suggesting that they act on the same population of neurones. Pretreatment of the neurones with BK, followed by its removal, resulted in a decreased response to a second application of the peptide. The extent of this decrease was dependent on the desensitizing concentration of BK (IC_50 value 30nM) and the duration of the pretreatment. Pretreatment with 30nM BK caused both a one-fold shift to the right of the concentration-response curve for BK-stimulated IP_3 formation (EC_50 increased to value 30nM) and a 50% reduction in the maximum response. Pretreatment with BK had no effect on subsequent responsiveness to serotonin, likewise pretreatment with serotonin did not affect the responsiveness to BK. After BK pretreatment, a prolonged incubation (60min) restored responsiveness of the cells to BK. Exposure of the cultures to dibutyryl cGMP or 4B-phorbol 12,13-dibutyrate (PDBu) also inhibited BK-induced IP_3 formation, causing a rightward shift of the BK concentration-response relationship and a decrease in the maximum response. These findings indicate that BK induces homologous desensitisation of inositol polyphosphate formation in DRG neurones which may involve cGMP-dependent kinase or protein kinase C.


We have previously reported that sigma ligands attenuate the ability of the cholinergic agonists, carbachol, to stimulate phosphoinositide (PI) turnover in rat brain synaptoneurosomes (Eur. J. Pharmacol., 169:399, 1988). Here we investigate the mechanisms of these effects. Sigma compounds were prelabelled with [3H]inositol, and [3H]inositol phosphates collected after exposure to twofold concentrations of (1)-pentazocine and (1)-tolyguanidine reduced the maximal stimulation by carbachol without affecting the EC_50, suggesting a non-competitive mechanism. Ligand concentrations 15-fold higher were required to attenuate norepinephrine-stimulated PI turnover. Although some sigma ligands inhibited [3H]inositol binding to muscarinic receptors at high concentrations, there was no correlation with potency in the PI assay. (1)-Pentazocine reduced formation of all three [3H]inositol phosphates and had no effect on their hydrolysis. When present during prelabeling, (1)-pentazocine did not affect incorporation of [3H]inositol into the total inositol lipid pool. The differentiation sensitivity of muscarinic and adrenergic stimulation, and lack of effect at other points in the PI pathway, suggests desensitization of muscarinic receptors.


The activation of adenine A(1) receptors in ADT1 smooth muscle cells resulted in a dramatic reduction of cyclic AMP (cAMP) accumulation. In addition to this reduction of cAMP accumulation, activation of 5-HT1 receptors decreased both (cAMP) accumulation and a 25-fold increase in norepinephrine-stimulated (NE-stimulated) PI turnover. While the adenine A(1) selective agonist, cyclopentyladenosine (CPA) had no effect upon PI turnover, it caused a 20-fold increase in norepinephrine-stimulated (NE-stimulated) PI turnover. To ascertain whether the effect of CPA on NE-stimulated PI turnover was related to the effect of CPA on cyclic AMP accumulation, 8-bromo-cAMP (8-BrcAMP) was added exogenously. While 8-BrcAMP had no effect upon NE-stimulated PI turnover, the CPA-induced stimulation of PI turnover was completely antagonized. The potentiation of PI turnover by CPA was also blocked by a monoclonal antibody which blocks the inhibition of cyclic AMP accumulation by CPA. In addition to being regulated by cAMP, the potentiation of PI turnover by CPA was calcium dependent and was inhibited by inhibitors of phospholipase A2 and by inhibitors of lipoxigenase. Thus, cAMP may regulate PI turnover by regulating the production of products of the lipoxigenase pathway which stimulate PI turnover. (HL-07502 and GM-31555)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

Increasing concentrations of KC1 (17.5 to 75 mM) caused concentration-dependent activation of protein kinase C (PKC) in rat anterior pituitary cells and sympathetic neurons (SN). Extent of PKC activation by 35 nM K+ was comparable to that seen with 100 nM phospholipase C. PKC activation was observed when 35 nM K+ was added to nerve terminals in the presence of 30 μM acetylcholine (ACh).

Biochemical pathway responsible for activation of PKC was studied in homogeneous cultures of chick embryonic SN 35 mM K+ did not cause an increase in PKC activity. In [3H]-inositol-loaded SN but produced a significant increase (160%) in 1,2-diacylglycerol (DAG) content. ACH enhanced PKC activity in a time-dependent manner (100%) and (DAG) content (165%). Turnover of [3H]-choline was facilitated by 35 nM K+ but not by ACH. Qualitatively similar results were obtained with 35 nM K+ and ACH on DAG and [3H]-inositol monophosphate formation in the rat adrenal medulla. These data suggest that depolarizing stimulus activates PKC in SN whereas ACH acts via phospholipidinositol pathway to produce DAG for activation of PKC.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

METABOLISM OF INOSITOL 1,3,4,5-TETRAKISPHOSPHATE IN RAT BRAIN HOMOGENATES. P. Kurian*, L. L. Chandler* and F.T. Krebs. Department of Pharmacology, Univ. of Florida College of Medicine, Box 100321, Gainesville, FL 32610.

A number of hormones, growth factors and neurotransmitters have been shown to stimulate the hydrolysis of membrane phosphoinositides, resulting in the formation of diacylglycerol and inositol phosphates. It is now clear that inositol phosphates is a complicated process that may be highly regulated. In addition to being dephosphorylated by the action of phosphatases, Ins(1,3,4,5)P_4 can be phosphorylated by Ins(1,4,5)P_3 kinase to form Ins(1,3,4,5)P_4. Although the physiological significance of the higher inositol polyphosphate is not completely understood, we have evidence that Ins(1,3,4,5)P_4 may also have second messenger function. To further understand the disposition of Ins(1,3,4,5)P_4, we investigated the metabolic pathways of this polyphosphate in rat brain homogenates in physiological buffers. Analysis of the inositol phosphate metabolites of Ins(1,3,4,5)P_4 by HPLC showed that [3H]Ins(1,3,4,5)P_4, the first dephosphorylated to Ins(1,3,4)P_3 and then to Ins(1,3)P_2 in a rat whole brain homogenates resuspended in physiological buffers. Analysis of the inositol phosphate metabolites of Ins(1,3,4,5)P_4 by HPLC showed that [3H]Ins(1,3,4,5)P_4, the first dephosphorylated to Ins(1,3,4)P_3 and then to Ins(1,3)P_2, produced by Ins(1,3,4,5)P_4 was formed. From these results, it was concluded that Ins(1,3)P_2 was detected, and then Ins(1,3)P_2 was formed from Ins(1,3)P_2, while Ins(4)P_2 formed from Ins(1,3)P_2 and/or Ins(1,3)P_2. These results support the idea that a gradient of second messengers that separates all of these isomers in a single run.
401. EFFECTS OF INTRATHRECALLY ADMINISTERED ISOQUINOLINESULFONAMIDES H7, H8 AND HA1004 ON ACOUSTIC STARTLE. R. Breglia and M. Davis. Dept of Psychiatry, Yale Univ, Sch. of Med., 34 Park St., New Haven, CT, 06510.

The spinal synapse of the acoustic startle reflex is subject to several forms of plasticity. The present study sought to evaluate the role of two second messengers at this site. The isoquinolinesulfonamide H7, and the ganglioside GTb, which inhibit protein kinase C activation, were infused intrathecally into the subarachnoid space of the lumbar spinal cord. H7 caused a marked, dose-dependent (10-300 µg) amplification of the startle reflex in comparison with its vehicle (p<0.01). Like H7, GTb (40 µg) produced a marked increase in the startle reflex versus its vehicle (p<0.05). HA1004 (150 µg) and H8 (150 µg), which have substantially less C kinase inhibitory activity than H7, caused only modest increases in startle. The isoquinolinesulfonamides have also been shown to inhibit a kinase activity. Of the group, H8 is the most effective. Previous work has shown that drugs thought to increase cAMP increase the startle response when infused intrathecally. In a second study, fifteen minutes after either H8 (130 µg) or vehicle infusions, animals received 50 µg of dibutyryl cAMP. By itself H8 had little effect on startle but completely blocked the normal excitatory effect of dibutyryl cAMP on startle (p<0.001). The pharmacological specificity of H8 was shown by the fact that dibutyryl cAMP continues to elevate startle in the presence of H7. Further, the inability of H8 to inhibit the excitatory effect of intrathecal 8-OH-DPAT infusions (p<0.19) demonstrates H8's behavioral specificity. Thus, while H8 inhibits a kinase-depends increase in kinase, it does not inhibit all increases in startle. Further, as evidenced by the primary increases in startle caused by intrathecal infusions of the isoquinolinesulfonamides and the ganglioside GTb, spinal PKC inhibition elevates startle, perhaps because PKC tonically inhibits startle at the spinal level.

CATECHOLAMINES: NEUROTOXICITY

402.1 NEUROTOXIC EFFECTS OF DSP-4 ON LOCUS COERULEUS (LC) AXONS IN THE RAT: IMMUNOHISTOCHEMICAL EVIDENCE FOR MORPHOLOGICAL CHANGES RELECTING DEGENERATION. J.-M. Fritschv*, Michel Geffard1, Michel Girard2 and R. Grzanna. 1) Dept. of Neurosciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 2) Laboratoire de Neuroimmunologie, CNRS - IBCN, 33077 Bordeaux, France.

Systemic injections of the neurotoxin DSP-4 produce depletion of noradrenaline (NE) and dopamine-1-hydroxylase (DBH) in brain regions innervated by the LC. We have studied the effects of DSP-4 on the morphology of central NE axons around a 2 week period using antibodies to NE and to DBH. Rats were sacrificed at daily intervals following a single dose of DSP-4 (1 p., 50 mg/kg; gift of Dr. S.B. Ross). One day after DSP-4, staining with anti-NE revealed extensive transmitter in LC axons. In contrast, there was a marked change in NE axon staining with anti-DBH until day 4. Thereafter, an abrupt loss of DBH staining was observed in brain regions innervated by the LC. This loss of LC axon staining was coincident with the appearance of morphologically altered axons. These fibers were characterized by their thick diameter and intense staining; some of these axons showed prominent swellings and had numerous short branches, resembling axonal sprouts. They were most frequent in the deep layers of cortex and along ascending pathways of NE axons. These remaining fibers could be visualized with both antisera between days 4 and 10. By day 14, there was no staining with either anti-NE or anti-DBH in brain regions innervated by the LC. We propose that the sequence of events following DSP-4 treatment can be divided into two phases: initially, there is rapid and profound loss of transmitter from LC axons; this phase is followed by morphological alterations of fibers and disappearance of staining. We suggest that the transient appearance of swollen axons and axonal sprouts and the loss of DBH staining mark the onset of LC axon degeneration. [Supported NIH grant MH 41977]

402.2 DSP-4 HAS DIFFERENT AFFINITIES FOR THE NOREPINEPHININE (NE) CARRIER IN CEREBRAL CORTEX AND HYPOTHALAMUS. R. Grzanna. R. Zapec, J.-M. Fritschv, S. Culp* and F.B. de Souza. Department of Neuroscience, Hopkins University School of Medicine and NIDA, AR, Baltimore, Md. 21205

A single systemic injection of the NE neurotoxin DSP-4 causes profound reductions in NE levels in both cerebral cortex and hypothalamus. It is not clear whether this neurotoxic effect is due to differences in the affinity of DSP-4 for the NE uptake system in these two regions. To test this, we measured NE uptake into synaptosomes isolated from these two areas in the presence of 100 and 300 nM DSP-4, respectively, and then determined the affinity of this uptake process (Ki) for DSP-4 using the Lineweaver-Burk method. We found that DSP-4 caused a larger reduction in NE uptake into cerebral cortex synaptosomes than into hypothalamic synaptosomes (Ki = 0.08 ± 0.03 nM versus Ki = 0.24 ± 0.04 nM, respectively). These results suggest that the differential neurotoxic effects of DSP-4 in these two brain regions may be related to differences in the affinities of the NE uptake carrier for the drug in these two regions.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes degeneration of the dopaminergic nigrostriatal pathway in experimental animals including C57Bl/6 mice. MPTP-induced neurotoxicity is dependent upon the monoamine oxidase-B (MAO-B) catalyzed formation of the 1-methyl-4-phenylpyridinium ion species (MPP+) from MPTP. Here, we measured neostriatal levels of MPP+ in naive female C57Bl/6 mice (3, 6, 9, and 18 months of age). Age-related effects of a single dose of MPTP (15 mg/kg, s.c.) were determined by measuring neostriatal levels of MPP+, dopamine and glutathione. Lower-neurotoxicants. The younger group had lower levels of MPO-B and GAP. After MPTP the 1-month old mice had 1) lower levels of neostriatal MPP+, 2) less depletion of neostriatal dopamine and 3) lower levels of striatal GAP in relative to the other groups. Data are consistent with previous reports of age-related MPTP neurotoxicity.


MPTP, a peripheral adrenergic depleting agent, substantially decreases mortality in the rat in response to large doses of MPTP (60 mg/kg s.c.) without directly affecting the neurotoxic actions of MPTP. In the present study, MPP+ was given in combination with guanethidine pretreatment to successively induce dopaminergic neurotoxicity in the rat. This treatment resulted in a marked depletion of dopamine (DA) and its metabolites and loss of tyrosine hydroxylase (TH) activity in the rat caudate nucleus. Additionally, MPTP induced a significant but slightly less pronounced decrement of DA and TH activity in the substantia nigra of the rat. The relationship between the loss of DA and TH activity in the caudate to the loss of 1) DA, 2) TH activity and 3) dopaminergic neurons in the substantia nigra will be discussed.


2′-ET-MPTP is, like MPTP, a potent dopaminergic neurotoxin. It has been proposed that 2′-ET-MPTP-promoted neuronal death is a consequence of 2′-ET-MPTP-induced inhibition of mitochondrial respiration. In the present study, we have examined the mechanism of 2′-ET-MPTP-induced toxicity in PC12 cells, which are neoplastic in nature and have a high rate of anerobic glycolysis with a large production of lactate and a low utilization of glucose carbon through the Krebs cycle. We compared the effects of varying concentrations of 2′-ET-MPTP on PC12 cells grown in RPMI 1640 medium supplemented with either glucose or with pyruvate. The latter substrate is directly metabolized via the Krebs cycle. 2′-ET-MPTP was more toxic to PC12 cells grown in the pyruvate-supplemented RPMI 1640 medium than cells grown in glucose-supplemented medium. For example after two days, 50 µM 2′-ET-MPTP killed 65% of cells in the pyruvate medium but only 1% of cells in the glucose medium. These results demonstrate that an alteration in the energy metabolism of the cells can affect their susceptibility to 2′-ET-MPTP. Results of these studies and reasons for this differential toxicity will be discussed.


The rat displays relative insensitivity to the neurotoxic actions of MPTP. The LD₅₀ of MPTP in the rat is lower than the dose necessary to produce neurotoxicity in the mouse. In the present study, MPTP was given in combination with guanethidine pretreatment to successively induce dopaminergic neurotoxicity in the rat. This treatment resulted in a marked depletion of dopamine (DA) and its metabolites and loss of tyrosine hydroxylase (TH) activity in the rat caudate nucleus. Additionally, MPTP induced a significant but slightly less pronounced decrement of DA and TH activity in the substantia nigra of the rat. The relationship between the loss of DA and TH activity in the caudate to the loss of 1) DA, 2) TH activity and 3) dopaminergic neurons in the substantia nigra will be discussed.


1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes degeneration of nigrostriatal dopaminergic neurons in monkeys. Monoamine oxidase-A (MAO-A) catalyzes the oxidation of MPTP to 1-methyl-4-phenylpyridinium ion both in vitro and in vivo, and this oxidation is a necessary step in the neurotoxic process. In vitro, MAO-A oxidizes MPTP analogs having either a methyl or ethyl group in the 2′position and also a role in the bioactivation of these compounds to neurotoxins in vivo. In these in vitro studies, we evaluated several analogs of MPTP to assess their abilities to be oxidized by MAO-A and MAO-B, and to cause nigrostriatal dopaminergic neurotoxicity in mice. Several were as evidenced by their capacities to cause reductions in the content of dopamine (DA) and its metabolites, binding of the DA uptake inhibitor in 1979, and tyrosine hydroxylase activity. Most of the neurotoxins were found to be bioactivated by MAO-A as well as MAO-B. Many of these compounds may turn out to be useful research tools.

402.10 NEUROCHEMICAL (CATACHOLAMINES) EFFECTS OF 6-OHDA LESIONS OF THE PRE-FRONTAL CORTEX IN 21 DAY OLD vs ADULT (9 WEEK OLD) RATS. V. Haroutunian, P.J. Knott, K.J. Davis*. Dept. Psychiatry, Mount Sinai School of Medicine, New York, N.Y.

Lesions of the dopaminergic innervation of the prefrontal cortex (PFC) affect the dopaminergic nigrostriatal system. In the present study, 21 days old and two week old (21 days old +1) rats received 25 mg/Kg DMI (IP) prior to the bilateral infusion of 6-OHDA (4ug in 1ul in weanlings and 8ug in 2ul in adults) into the striatum of the PFC. Levels of norepinephrine, dopamine and homovanillic acid were measured in the PFC, caudate nucleus (CPU), and n. accumbens (ACB) 7 days later. DA and NE in the PFC were significantly (p<0.01) reduced in both age groups. Subcortically, levels of dopamine in the ACB were increased by 220% in weanling rats relative to the 142% increase seen in adult rats. DA activity in the CPU was unchanged. These data suggest that ontogenetic lesions of the PFC may lead to greater perturbation of subcortical DA function than similar lesions sustained in adulthood.
402.11


Extracellular single unit recording and microiontophoresis techniques were used to determine the sensitivitiy and interaction of D1 and D2 receptors in the CPu of rats which were pretreated with either 6-OHDA (100 g/5 ml, LV), 6-OHDA plus AMPT. Seven-ten days after 6-OHDA, CPu DA levels were reduced by 88%. Cells/tracks analysis revealed more spontaneously active CPu cells in 6-OHDA-lesioned rats. Current-response curves exhibited significantly enhanced inhibitory responses of CPu cells to both the D1 agonist SKF 38393 (SKF) and D2 agonist quinpirole (Quin). However, the potentiation of Quin-induced inhibition by co-injected SKF observed in controls was abolished by 6-OHDA pretreatment. Moreover, the absolute increases in current for SKF to evoke Quin’s effect in unlesioned rats acutely depleted (80%) of DA with AMPT was also eliminated by 6-OHDA (with and without AMPT). These findings indicate that 6-OHDA lesions result in (1) the functional sensitization of both D1 and D2 receptors and (2) the loss of D1-mediated enabling of D2 responses in the rat CPu (Supported by USDA, USPHS Grants DA-04093, and MH-40392 to EJW).

402.12


Lesions of nigral dopaminergic cells with 6-hydroxydopamine (6-OHDA) cause increases in proenkephalin (PEK) mRNA but decreases in protachykinin (PTK) mRNA in rat striatum (Angulo et al. 1983). In the present study, we have investigated the possibility that nerve growth factor (NGF) may influence these changes. Adult rats received unilateral 6-OHDA-induced nigral lesions. The animals were then tested until they developed stable apomorphine (APO)-induced rotation. They were then divided into two groups according to rotational behavior and were given intraventricular infusions of either NGF or saline for 28 days. The rats were then sacrificed and the brain processed for in situ hybridization histochemistry. As previously reported, 6-OHDA nigral lesions caused increases in PTK mRNA but decreases in PTK mRNA in rat striatum. These changes were potentiated by chronic infusion of NGF. The potentiation was more evident in the dorsolateral aspect of the caudate putamen. However, in situ hybridization for tyrosine hydroxylase (TH) mRNA in the SN showed no differences between the groups. The data will be discussed in relation to the topography of striatal dopamine afferents from the SN.

402.13

RESEARCH LABORATORIES, Research Triangle Park, NC 27711.

MPTP induced damage to substantia nigra neurons has been hypothesized to occur as a result of axonopathy followed by retrograde degeneration. To address this issue we have used glial fibrillary acidic protein (GFAP) and tyrosine hydroxylase (TH) immunohistochemistry as well as a silver degeneration stain to characterize alterations occurring at the terminal fields within the striatum and neurons within substantia nigra. C57 mice were given 12.5, 20 or 40 mg/kg MPTP and sacrificed at intervals from 12 hours to 5 days postdosing. Marked increase in GFAP immunoreactivity occurs in stratum of MPTP animals at times when only slight increases were observed in substantia nigra. Increases in GFAP parallel decreases in TH immunoreactivity and evidence of silver degeneration. These results further characterize the vulnerability of striatal axons to MPTP.

CATECHOLAMINES III

403.1

COMPARISON OF THE PHARMACOLOGICAL RESPONSIVENESS OF Dopamine in the Rat Medial Prefrontal Cortex and Striatum. B. Mochaddam and B.S. Bunney, Dept. of Pharmacology & Psychiatry, Yale University School of Medicine, New Haven, CT. 06510.

Using the techniques of in vivo microdialysis combined with a small-bore liquid chromatography system, we have measured the basal and drug induced fluxes of extracellular dopamine (DA) in the medial prefrontal cortex (mPFC) of chloral hydrate anesthetized rats and have compared our findings in the mPFC to those observed in the striatum. The results were as follows: (1) At a flow rate of 2 µl/min, basal level of DA in the mPFC, was 0.32±0.1 (n=28) femtomoles/µl perfusate, which was nearly an order of magnitude less than that obtained from striatum, (2) Behaviored µ-t-methyl-β-tyrosine (AMPT, 500 mg/kg, ip) or 6-OHDA plus AMPT. Seven-ten days after 6-OHDA, CPu DA levels were reduced by 88%. Cells/track analysis revealed more spontaneously active CPu cells in 6-OHDA-lesioned rats. Current-response curves exhibited significantly enhanced inhibitory responses of CPu cells to both the D1 agonist SKF 38393 (SKF) and D2 agonist quinpirole (Quin). However, the potentiation of Quin-induced inhibition by co-injected SKF observed in controls was abolished by 6-OHDA pretreatment. Moreover, the absolute increases in current for SKF to evoke Quin’s effect in unlesioned rats acutely depleted (80%) of DA with AMPT was also eliminated by 6-OHDA (with and without AMPT). These findings indicate that 6-OHDA lesions result in (1) the functional sensitization of both D1 and D2 receptors and (2) the loss of D1-mediated enabling of D2 responses in the rat CPu (Supported by USDA, USPHS Grants DA-04093, and MH-40392 to EJW).

403.2

INTRACELLULAR STUDY OF THE EFFECT OF NOREPINEPHRINE ON SOMATOSENSORY CORTICAL NEURONAL RESPONSE TO GABA. W. Lin, P.M. Sessler, C.S. Lin and B.D. Waterhouse, Dept. of Physiology and Biophysics, Bowmanman Univ., PA. 18710-1192.

Considerable evidence from extracellular recording studies suggests that norepinephrine (NE) may augment inhibitory synaptic transmission within local circuits of mammalian neocortex. For example, experiments in intact animals have shown that microiontophoresis of NE or activation of the cortico-cortical afferent pathway can enhance stimulus evoked or GABA-induced depressant responses of cortical neurons. Further studies using neocortical tissue slices have demonstrated that these noradrenergic modulatory actions involve the beta-adrenoceptor linked cyclic-AMP second messenger system. In the present investigation we have begun to examine the transmembrane electrophysiological events which are associated with NE and GABA interactions on somatosensory cortical cells. Intracellular recordings were made from somatosensory cortical neurons in a submerged tissue slice preparation. The resting membrane potential of the cortical neurons (N=24) examined was 68.7±8.6 mV, with an input resistance of 33.1±12 megohms. In four of five cells tested, superfusion of NE at concentrations producing little or no change in membrane potential was associated with an increase in input resistance. In 40% of the cases tested, superfusion of NE during GABA application produced a net increase in membrane conductance, suggesting noradrenergic facilitation of GABA-mediated membrane conductance activity.

Recent studies have indicated that both β1 and β2 adrenoceptors are present in rat neocortex and can alter various aspects of neuronal excitability in vitro. In these studies, we have utilized extracellular recording to examine the effects of α2-adrenoceptor agonists and antagonists on the excitability of mean- and depression-sensitive neurons. The results of these experiments suggest that the α2-adrenoceptor agonist clonidine is capable of depressing the excitability of neurons in a manner that is dependent upon the stimulus strength used to elicit the response. These findings are consistent with the hypothesis that α2-adrenoceptors play a role in the regulation of neuronal excitability in the neocortex.


To investigate the role of α2-adrenoceptors in the regulation of nociceptive transmission in the spinal cord, we have studied the effects of the selective α2-adrenoceptor agonist clonidine on the evoked responses of Aδ and C fibers in the spinal cord of the newborn rat. Our results indicate that clonidine reversibly depresses the evoked response of Aδ fibers, while having no effect on the evoked response of C fibers. These findings suggest that α2-adrenoceptors may play a role in the modulation of nociceptive transmission in the spinal cord of the newborn rat.

MICROINJECTION OF LIDOCAINE, GAHA, OR SYNAPTIC DECOUPPLERS INTO THE VENTROLATERAL MEDULLA BLOCKS SCALED-EVOKED ACTIVATION OF LC IN RATS. Charles H. K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Joseph E. Lowery Boulevard, Atlanta, GA 30307.

In this study, we have investigated the role of the ventrolateral medulla in the modulation of noradrenergic activity in the locus coeruleus (LC). Our results indicate that microinjection of lidocaine or glycine (GABA) into the ventrolateral medulla selectively blocks the evoked activation of LC in rats. These findings suggest that the ventrolateral medulla plays a role in the regulation of noradrenergic activity in the LC.

RESPONSES OF MESOLIMBIC UNITS TO SENSORY INPUT IN RATS CONDITIONED BY REINFORCING BRAIN STIMULATION. Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Joseph E. Lowery Boulevard, Atlanta, GA 30307.

In this study, we have investigated the effects of conditioning brain stimulation on the responses of mesolimbic units to sensory input. Our results indicate that conditioning brain stimulation selectively increases the responsiveness of mesolimbic units to sensory stimuli, and that this effect is mediated by α2-adrenoceptors.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
MUltiple projection pathways from the ventrolateral medulla to the nucleus coeruleus in rat. E.J. Van Bockstaele, B. Astier*
We have previously found that VLM (nucleus paragangocellularis) is a major afferent to locus coeruleus (LC). Here, we examined afferent projections from this area using Phycocyanin (Phc) and Fluoro-Gold (FG) retrograde labeling. Anterograde labeling was identified in LC as well as many other brain nuclei. We examined the projection from VLM to LC in detail to explore possible topographies of innervation in LC and determine the VLM nuclear group(s) providing this input. Several anterograde labeling techniques were used and the results allow us to conclude that VLM projecting cells are distributed throughout VLM and includes both the lateral and caudolateral portions of this nuclear group. These results indicate that VLM may provide sparse innervation of LC. Support: PHS grants NS20643 and NS24968.

Nissl, Golgi and immunocytochemical (ICC) studies have described the morphology and ultrastructure of rat locus coeruleus (LC). However, little is known about LC neuronal processes extending outside of the nucleus proper. Here, ICC LM and EM techniques were used to examine the distribution of TH- and DBH-labeled extraneuronal processes in the pericellular region. DBH-labeled processes preferentially extended into the rostromedial pericellular region. Thus, it may be that the LC neuronal morphology in rostral and medioventral portions of LC is different from that in the rest of the nucleus. Some labeled processes extend caudally but very few appear to extend rostrally and laterally. In horizontal sections it is clear that even neurons in the rostral and medioventral regions of LC contain a high density of TH- and DBH-labeled processes. These results indicate that VLM projections to LC may derive from a more widespread area of VLM than previously reported. EM-ICC analysis revealed that of 160 TH- or DBH-labeled profiles identified in the rostral and medioventral pericellular region all were dendritic. Labeled axons were not observed. These results indicate that the dendrites of LC neurons tend to be more extensions rather than terminal ramifications of the neuronal soma. The vast majority of extraneuronal LC dendrites extend and ramify in the rostromedial pericellular region. The rostromedial pericellular region may be a site for regulation of LC. (Supported by PHS Grants NS20643, NS 24968 and NS23348.)

The dopamine (DA) neuronal projection to the nucleus accumbens (NAc) is important in mediating the reward-related effects of cocaine (COC). We have recently reported that NAc neurons are supersensitive to the inhibitory effects of l- norepinephrine (NE) and paragangocellularis, terminate in LC proper and also the pericellular region medial and rostral to LC. Recent studies demonstrate dense GAARergic and peptidergic terminals in rostral and medial pericellular region. Thus, this pericellular region may be a key site for regulation of LC. (Supported by PHS Grants NS20643, NS 24968 and NS23348.)

Recent studies have revealed that locus coeruleus (LC) receives only a few dopaminergic inputs. The influence of LC on the mesolimbic neuronal system has been difficult to ascertain. Here, we examined possible projections from one area, periaqueductal gray (PAG), recently reported to project to the subcortical (LC). PHA-L injections in PAG laterally adjacent to the accumbent labeled fibers that profusely innervate the region rostral and medial to LC, and nucleus paragangocellularis. Here, we examined possible pathways from VS to LC. In agreement with these anatomical results, focal electrical stimulation of LC produced sparse antidromic activation in PAG. (N=34) LC neurons were antidromically driven at latencies of 4-6 ms. These driven cells did not respond to electrical stimulation of the hippocampus, a manipulation that activates LC neurons. Stimulation of PAG (5 Hz) synaptically activated 60% of LC cells tested at onset latencies of 4-10 ms. Such excitation was weak and typically required high currents (>500 uA). Stimulation of PAG lateral to the acqueucted activated 77% of LC neurons usually at shorter latencies (4-7 ms), similar to those for PAG neurons antidromically driven from LC. Stimulation of ventrolateral PAG yielded antidromic or pure inhibitory responses. These results indicate that PAG may provide sparse innervation of LC. Supported by PHS Grants NS20643 and NS24968.

D, and D2 dopamine receptor agonists decrease inwardly rectifying currents in the central nucleus of the rat amygdala. M.C. Schier and P. Shinnick-Gallagher. Dep. Pharmacology, Univ. Texas Medical Branch, Galveston, TX 77550.
The membrane activity of dopamine (DA) and the specific D2 agonist SKF 38393 (SKF) and the D2 agonist Quinpirole (Quin) was studied at a postsynaptic projection site, the central nucleus of the rat amygdala (ACe). Micromolar concentrations were superfused onto 500 micron thick in vitro slice preparations and intracellular membrane activity was recorded under voltage clamp. There were two multipletype classes of cells with two distinct fiber pathways from rostral VLM to LC. These results reveal 3 separate pathways from the VLM to LC. Additional studies are needed to characterize neurons projecting to LC by these pathways. Supported by NINDS grant NS24698 and ONR/AFOSR contract N00014-86-K-0493.

We have previously demonstrated that impulse-regulating autoreceptors on A10 dopamine (DA) cells in the rat ventral tegmental area become subsensitive to DA and DA agonists following repeated administration of cocaine. The present study sought to define if this subsensitivity persists following cocaine withdrawal. Male rats were given twice daily i.p. injections of 10 mg/kg cocaine or saline for 7 days. Three groups were then tested at various times after cocaine withdrawal: 1, 4 or 7 days. Extracellular single-unit recording techniques were used to determine the sensitivity of somatodendritic autoreceptors on A10 DA cells as indicated by the inhibitory effects produced by i.v. apomorphine (APO). As previously reported, A10 DA neurons were subsensitive to APO 1 day after the last injection of cocaine. Following 4 days of withdrawal, subsensitive responses to APO were still observed, although the extent of subsensitivity was reduced. With further periods of withdrawal, subsensitive responses to APO were still observed, although the extent of subsensitivity was reduced. With further periods of withdrawal, subsensitive responses to APO were still observed, although the extent of subsensitivity was reduced. With further periods of withdrawal, subsensitive responses to APO were still observed, although the extent of subsensitivity was reduced.
MORPHINE WITHDRAWAL ACTIVATION OF LOCUS COERULEUS NEURONS: ATTENUATION BY LESIONS OF NUCLEUS PARAGANGLIOCELLULARIS. T.C. Napier. Dept of Pharmacol, Loyola Univ of Chicago, based upon certain electrophysiological characteristics.

Additionally, these cells had action potentials which were suppressed by DA had a slower firing rate (18.8—1.9 Hz). Differentially to Microiontophoretically Applied Dopamine.


After NA or 5-HT applications, the acetylcholine-induced excitation was suppressed or decreased in 60% of the cells tested. These results provide evidence of widespread effects of monoamines on S-I neurons in awake rats. But also of laminar specificity.

THE FIRING RATE OF VENTRAL PALLIDAL NEURONS IS AFFECTED BY DOPAMINE AGONISTS. R.J. Malouwski* and T.G. Napier, *Med., Maywood IL 60153. The ventral pallidum (VP) receives monosynaptic inputs from a dopaminergic region, the ventral striatum. Thus, activation of dopaminergic neurons in the ventral striatum may also affect VP neuronal activity. The activity of VP neurons was assessed following systemic injection of apomorphine, a nonselective dopamine receptor agonist. The firing rate of 82% of the VP neurons tested (n=34) was affected by apomorphine. The responses were equally distributed between increases and decreases in firing rate. SKF38393 (0.1 mg/kg, iv) blocked 70% of the apomorphine-induced responses (n=16). Sulpiride (32.5mg/kg, iv), a D2 antagonist, reversed the responses in 50% of the cells (n=12). Subsequently, the effects of the D1 agonist, SCH23390 (0.1mg/kg, iv) and a D2 agonist, quinpirole (0.025-0.05 mg/kg, iv), were also studied. These results provide further evidence for the presence of D1 receptors on dopaminergic axons in the neostriatum. (Supported by NIDA.)

ALTERATIONS IN CONCENTRATIONS OF 3-METH0XY-4-HYDR0XY-PHENYLETHYLENYGLYCOL (MHPG) IN THE PARAVENTRICULAR NUCLEUS (PVN) AND SUPRAOPTIC NUCLEUS (SON) REFLECT THE ACTIVITY OF NORADRENERGIC (NE) NEURONS PROJECTING TO THESE HIPOTHALMIC REGIONS. K.J. Loomingland, L. Ireland*, Y. Tian*, J. Manganas and K.E. Moore, Dept. of Pharmacol/Toxicol, Michigan State Univ., East Lansing, MI 48824.

In the rat brain MHPG, a major metabolite of NE, exists predominantly as a conjugate and, as such, cannot be quantified using HPLC. Therefore, this study was conducted to determine MHPG concentrations in the PVN and SON procedures that activate NE neurons. MHPG was not detectable in untreated samples, but following acute hydrocortisone, significant amounts of MHPG were measured in the PVN and SON. Activation of NE neurons by physical restraint or the administration of the α2-adrenoceptor antagonist idazoxan increased MHPG concentrations in both the PVN and SON. On the other hand, electrical stimulation of the locus coeruleus increased MHPG concentrations in the PVN, but not in the SON. These results indicate that procedures that activate NE neurons originate in sub-coeruleus regions of the pons-medulla. These results indicate that procedures that activate NE neurons originate in sub-coeruleus regions of the pons-medulla.
403.21

BEHAVIORAL SENSITIZATION TO APOMORPHINE DEVELOPS THROUGH BOTH ASSOCIATIVE AND NONASSOCIATIVE PROCESSES. B. A. Nettingly and J. E. Gotsch†, Department of Psychology, Morehead State Univ., Morehead, KY 40351.

In three experiments, male rats received intermittent SC injections of apomorphine (APO:5.0 mg/kg) or vehicle (VH) and were tested for locomotor activity. In Exp. 1, rats received either nine paired or unpaired exposures to APO and the test environment. In Exp. 2, rats were either tested for activity or returned to their homcage following each of ten APO or VH injections. In Exp. 3, rats were given either daily VH injections or APO and VH injections alternated daily for 26 days. Following the pretreatment phase in each experiment, all rats were tested for activity following an APO injection. Major findings were: (a) both paired and unpaired APO exposure produced behavioral sensitization, but the magnitude of the sensitization effect was greater for the paired group; (b) rats sensitized to APO displayed no evidence of conditioned hyperactivity when tested following a VH injection; (c) rats pretreated with APO without activity testing also demonstrated significant sensitization when subsequently tested for activity; and (d) significant sensitization to APO was observed when neither test apparatus cues nor injection/handling cues were reliably associated with APO treatments. These findings indicate that behavioral sensitization to APO develops through both associative and nonassociative processes.

404.1


LHRH neurons originate in the olfactory placode and migrate into the forebrain along branches of the terminals and vomeronasal nerves (Nature, 1989, 338:161-164). The present study examined, in mouse, the ultrastructure of these LHRH-neurons during migration and found that the subcellular location and density of LHRH-immunopositive product varied with the location of the LHRH-neurons along the migratory route. LHRH-neurons, first detected at E 11 in the epithelium of the medial part of the olfactory placode just before migration, showed LHRH-immunopositive product heavily accumulated around the outer nuclear envelope and in the lumen of the rough endoplasmic reticulum (rER) adjacent to the nucleus. From E 11.5 through E 14, LHRH-immunopositive cells were seen in cords on the nasal septum, with LHRH-immunopositive product heavily accumulated around the outer nuclear envelope and in the lumen of the rER. At E 16, no LHRH-immunopositive cells were seen in the olfactory placode just before migration, showed LHRH-immunopositive product heavily accumulated around the outer nuclear envelope and in the lumen of the rER. At E 16, no LHRH-immunopositive cells were seen in the olfactory placode just before migration, showed LHRH-immunopositive product heavily accumulated around the outer nuclear envelope and in the lumen of the rER. By E 18, LHRH-immunopositive cells were seen in the forebrain and LHRH-immunopositive neurons formed denseplexuses in the OVLT and in the ME. Supported by NIH grant 18962 and the Whitehall Foundation.

404.2


Recent studies demonstrated, that perinatal ablation of the olfactory tubercle precede the development of hyperactive behavior in the C and B strain, but not in the 1 strain. The reason why the perinatal ablation of the olfactory tubercle precedes the development of hyperactive behavior in the C and B strain, but not in the 1 strain, is not clear. In this study, we wished to address the question whether the perinatal ablation of the olfactory tubercle would have a similar effect in the 1 strain.

In three experiments, male rats received intermittent perinatal olfactory tubercle ablation (Vadasz, Cs. et al.; Hormones and Behavior, 22:528, 1988). Here we wished to address the question whether this 'critical period' is restricted to perinatal development, or neonatal orchectomy would also have strain dependent organizational effects on brain dopamine system.

Male offspring of three inbred mouse strains (BALB/cJ - C; C57BL/6J - B; C57BL/6J - I), known to have high (C), medium (B6) and low (1) TH activity, were gonadectomized within 24 hours after birth (25-28 animals per strain), and sham operated at adult age. Males of the same strain castrated at adult age served as controls.

Day-1 gonadectomy tended to lower TH activity in all brain areas and had a "fear-reducing" effect on open-field behavior in the C and B6 strains, however these variables were not affected significantly in the 1 strain.

The results provided evidence that in mice (1) the developmentally sensitive period of brain dopamine systems in terms of TH activity includes early postnatal development and (2) the "sensitivity" to testosterone deprivation by Day-1 orchectomy is genotype dependent.

404.3

MORPHOMETRIC ANALYSIS OF CULTURED HYPOTHALAMIC TYROSINE HYDROXYLASE AND NEUROPHYSIN CELLS. M. Morrise*, B.A. Bennett, J. Giedelkars, D.W. Pfaff and M. Schwallen-Fukuda, Dept. of Physiology and Pharmacology, Wake Forest University Medical Center, Winston-Salem, NC 27103.

Studies were performed to characterize the effect of a depolarizing KCl stimulus on specific hypothalamic cell types. Hypothalamic cultures were stained immunohistochemically for neuron specific enolase (NSE), neurophysin (NP) or tyrosine hydroxylase (TH) and neuronal density and size were quantitated. The cultures were prepared from neonatal hypothalami which were enzymatically dispersed. Control or KCI treated (25 mm) cultures were fixed after 7 days and stained for NSE, NE or TH. KCl caused a universal increase in cell size. The cell area was increased from 99.4 and 123 mm² in the controls to 140.1 and 163.7 mm² in the KCl cultures (NP and TH, respectively). A change in the pattern of NP cell size was also noted. The KCl treatment resulted in a significant proportion of large NP positive cells (144.2 vs. 84.5 mm²). With regard to cell density, the most marked effect was on the NSE population. The cell density was reduced to 95% as compared to 100% in the KCl treated cultures. There was also an increase (29%) in the number of NP positive cells, while the density of TH cells was not altered. This discrepancy suggests that treatment with a depolarizing stimulus has specific effects on cultured hypothalamic neurons. It enhances survival of the total neuron population as well as increasing the size of TH, NP and NSE cells.

404.4


The development of catecholaminergic (CA) neurons in the embryonic chick brain has been investigated previously by fluorescent histochemical methods. Here, we have compared the results of these earlier studies with an examination of the ontogeny of CA neurons investigated by anti-TH immunocytochemistry. TH-containing cells were demonstrated by the peroxidase ABC method of staining paraffin sections. Embryos were examined at intervals of 5, 7, 9, 15 and 21 (E21) days of incubation.

The earliest immunoreactive cells were found at E5. Numerous immature TH-immunonegative neurons were present in rostral portions of the lateral hypothalamus. By E7, at least 3 distinct groups of deeply-stained cells were observed throughout the hypothalami, while also staining was detected in a small number of cells within the region of the developing substantia nigra. By E10, the majority of CA cell groups defined histochemically in the adult chicken were recognizable in the E9 embryo. By E15, the mature distribution of CA cellular subfields in the various brain regions of CA cell nuclei were clearly evident. The results of our immunocytochemical study demonstrate the presence of TH in neurons of the early chick embryo brain that far precedes the earliest histochemical detection of CA in these cells. Overall, though, the differentiation of CA nuclei appears to lag far behind that of central serotonergic systems. Funded by NIH grants RR-08139 and BRSG award SO7-RR05583-24.
404.5


Embryos of the fresh water snail, Helisoma trivolvis, are accessible to developmental and behavioral studies throughout embryogenesis and into larval life. The neuroanatomical and behavioral criteria described in this study have established this as a useful model system for comparing neural development with adult plasticity and regeneration. The objectives of this study were (1) quantitatively describe the stages of embryogenesis and the temporal sequence of ganglionic differentiation. Morphological and behavioral criteria were used to define embry stage, which were expressed as a percentage of the duration of embryogenesis. Ganglionic differentiation was analyzed using light and electron microscopic techniques. Cilia-driven spinning, the first recognizable behavior, began at 20% of embryonic development (stage E20). Other embryonic behaviors and their onset were cardiac beating, head wall contractions (both at E65), ciliary crawling (E80) and coordinated buccal rasping (E70). Ganglionic differentiation also began at E20, with the formation of the cerebral ganglia. Next to form was the pedal ganglia, at E25, followed by the buccal ganglia (E30), the pleural ganglia (E35) and finally, the parietal/visceral ganglia at E60. Morphologically, the characteristic veliger and juvenile forms appeared at E30 and E50, respectively. 

Supported by NSF BNS 8820658 and NIH 32978.)

404.6

HELISOMA EMBRYOGENESIS: MORPHOLOGICAL, BEHAVIORAL, AND NEURAL DEVELOPMENT. K. McKenzie and J. J. Goldberg, Dept. of Zoology, University of Wisconsin, Madison, WI 53706. NSF-BNS-881020, NSF-BNS-8805138 and by the FIPF program of the U.P.R.

H. trivolvis nauplii 4 days after hatching were transferred to a medium containing a mixture of 5-HT and/or 5-HT uptake inhibitor were added. Sites of 5-HT uptake or synthesis and 5-HT localization were then identified using immunocytochemistry. 5-HT immunoreactivity (5-HT-IR) was observed in the adult peripheral nervous system, in the brain, in the sense organs, in the digestive tract and in the central nervous system of the adult. 

These findings provide evidence that 5-HT may have a role in the development of the peripheral nervous system in H. trivolvis. 

Supported by NIH grant 5T20.EB01412.
MODULATION OF ADRENERGIC EXPRESSION BY THE TRICYCLIC ANTIDEPRESSANT DESIPRAMINE (DMI). M. Sieber-Blum and J.-M. Zhang. Department of Anatomy and Cellular Biology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Catecholamine fluorescence (FIF) in clonal cultures of quail neural crest cells is suppressed by norepinephrine uptake inhibitors (Sieber-Blum & Silverman, Neurosci. Abstr. 11, 806, 1985). We here report that transport of tritiated NE was developmentally regulated and that the inhibitory effect was reduced when the addition of DMI (10μM) was delayed, indicating that the cells were most sensitive during the early stages of their in vitro development. Exposure to DMI for 3-day time blocks at various stages of development did not significantly decrease the number of colonies containing FIF-positive cells (p > 0.57). However, continuous exposure to DMI caused a pronounced decrease in the number of tyrosine hydroxylase and 3β-hydroxysteroid (p = 0.0006) immunoreactive cells. These data suggest that a) DMI acts before overt expression of the adrenergic phenotype, b) DMI is nontoxic, c) a chronic exposure is necessary, and d) the drug alters catecholaminergic enzyme levels. We conclude that the NE uptake system may play a role in adrenergic development, and that tricyclic antidepressants may influence adrenergic neurogenesis during embryonic development. Supported by USPHS grant HD21423.

THYROID HORMONES ALTER THE POSTNATAL DEVELOPMENT OF THE 68 KD NEUROFILAMENT PROTEIN IN THE CEREBRAL CORTEX: AN IMMUNOCYTOCHEMICAL ANALYSIS. Nancy J. Wolff, Louise D. Ott, and Larry J. Bauer, Laboratory of Chemical Neuroanatomy, Dept. of Psychology, University of California, Los Angeles, CA 90024-1563, U.S.A.

Rat pups were made thyrotoxic by administering 1 mg/kg triiodothyronine i.p. daily. Other pups were made hypothyroid by providing 0.3% propylthiouracil in the diet of the dam during gestation and the neonatal period. At 1 week to 4 months postnatal age, the rats were sacrificed at 1, 2, 3, and 4 postnatal weeks. Immunostaining for NF 68 was observed in pyramidal cells throughout the cerebral cortex. In the 1 week postnatal brain very weak NF 68 staining was observed. At 2 weeks postnatal the NF 68 immunoreaction product was increased in intensity and was concentrated in the perikarya and proximal apical and basilar dendrites. At 3 and 4 weeks postnatally, the immunoreactivity was greater in the distal apical and basilar dendrites of cortical pyramidal cells and less product was found in the cell body as compared to the second postnatal week. Thyroid deficient rat pups showed markedly fewer dendrites as compared to rats containing thyroxine and hyperthyroid pups. Moreover, hypothyroidism retarded the appearance of NF68 in distal dendrites during development. At 3 and 4 weeks NF68 was observed in proximal and basal dendrites and cytoplasm. [Support: USPHS grant NS 10928 to L.L.B.]

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THE PRENATAL EFFECTS OF SKF 38393, A D-1 AGONIST, ON THE BIOCHEMISTRY AND BEHAVIOR OF NEONATAL RAT. A. Shenker and P.M. Whittaker-Atmila. Dept. of Psychiatry, SUNY at Stony Brook, NY 11794.

We have developed an in vivo model to assess the role of dopamine agonists on the development of dopamine synapses. In our present study we administered the D1 agonist SKF 38393 1mg/kg to pregnant Sprague Dawley rats from D12 of gestation to parturition. The resultant offspring showed lasting changes in the high affinity uptake of 3H-DOPA, indicative of terminal density and in the binding to spiroperidol. On PND 15 the treated neonates were less active in the open field and alternated less than their controls in a spontaneous alternation paradigm. At 90 days neonates were trained in a fixed ratio operant task and, when challenged with serotonergic drugs and dopaminergic drugs, showed significantly less sensitivity to both classes of drugs than did control neonates. The presence of a dopaminergic agonist during gestation produced changes in both the serotonin and the dopamine system which indicates an interaction between the two systems during development, causing a compensatory increase in the outgrowth of the unaffected transmitter system.

POST-MITOTIC NEUROMUSCULAR DIFFERENTIATION OF CREATINE KINASE IN POST-NATAL DEVELOPING RODENT MUSCLES. J.P. Shanahan, G. Licea, and E. Jimenez, Depto. de Bioquimica, Centro de Investigacion y de Estudios Avanzados-I.P.N., Mexico, D.F.

Creatine kinase mRNA in rat post-natal muscle development. In rat extracts of white muscle, brain, red and heart muscles were found to be present in the proteins isolated from rat post-natal muscle development. In contrast to the previous report, skeletal muscle creatine kinase (CK) activity was shown to increase in a dose dependent manner. The presence of a dopaminergic agonist during gestation produced changes in both the serotonin and the dopamine system which indicates an interaction between the two systems during development, causing a compensatory increase in the outgrowth of the unaffected transmitter system.

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405.5
THE RAT PUP ULTRASONIC ISOLATION CALL: STUDIES OF SEROTONERGIC AND ADRENERGIC NEUROTOXINS. I.T. Winslow and T.R. Isack. Lab. Clin. Sci., NIMH, NIHAC, Bethesda, MD. 20892. Rat pups removed from their nest and siblings emit a highly stereotyped and well characterized isolation call in the ultrasonic range. This behavior is selectively sensitive to the monoamine agonists and antagonists, and monoamine reuptake inhibitors. To further characterize the role of serotonin and norepinephrine transmission in the postnatal development of social behavior, selected doses of MDMA were administered subcutaneously to pups beginning 24 hr after birth. 10 mg/kg MDMA was administered once or twice daily, for 4 days. 50 mg/kg D- or L- (+)-amphetamine was administered once daily. To further characterize the experimental isolation design, both DSP-4 and vehicle control pups were injected with saline twice daily for 4 days, and pups in all treatment groups were tested 6, 9, 12, and 15 days postnatally. Body weight and number of pups recovered significantly increased in both D- or L- (+)-amphetamine treated groups compared to saline treated controls. Supported by grants HD20327, TOF/SAT, NS24386-JEC and HD0147-MR.

405.6
EARLY DEVELOPMENT OF THE CHOLINERGIC SYNAPSE IN RAT AND HUMAN BRAIN. A. Biegon, O. Bar-Feld and M. Segal. Weizmann Institute of Science, Rehovot, Israel. The ontogeny of Acetylcholinesterase (AChE), muscarinic and nicotinic cholinergic receptors was investigated by quantitative histochemistry (Biegon, A. and Wolff R., J. Neurosci. Methods 16: 337, 1986) and autoradiography (Biegon et al., T, Neurochem. 51:1381, 1988) in rats from day 16 of fetal life and in human abortuses (16, 18 and 24 weeks gestation). Each material was utilized to classify a specific developmental pattern. Rat AChE activity increases very slowly in cortex, striatum and hippocampus and is still very low compared to adult levels on day 18. By day 21, Cholinergic (e.g. medial septum) nuclei reach adult levels much earlier, between 7 and 14 days PN. In the human fetus, too, adult levels in mesial temporal structures (substantia innominata by the 24th gestational week, while cortical, hippocampal and striatal activity is very low. Nicotinic (n-bungarotoxin binding) is high in the earliest ages tested in rat and human hippocampus, followed by a decline towards adult levels. Muscarinic receptors in both species increase monotonously with age in most regions, reaching adult or higher levels by day 14 PN in rats and week 24 in humans. High receptor levels preceding the appearance of a presynaptic cholinergic innervation may play a developmental role in the cholinergic synapse.

405.7
RETIROAD REGULATION OF GENE EXPRESSION IN THE EARLY NERVOUS SYSTEM. S.G. Chen, L. Luomi, A.K. Hall, J. Hempstead, R.Zajic, and J. MorganSPON: R. Wurzburger. Deprt. of Neuroscience. Roche Institute of Molecular Biology, Nutley, N.J. 07110. Using HPLC techniques we have identified polypeptides whose expression, (a) alters during normal neuroembryogenesis and (b) are regulated by retinoid acid in neural cell cultures. Seven candidate molecules have been isolated, sequenced and their mRNAs and genes cloned by recombinant DNA techniques. A 43 amino acid polypeptide, termed thymosin beta 10, was found to be very abundant in the early rodent nervous system. Following gel electrophoresis and immunoblot procedures antibody 3D10 appears to react with a single high molecular weight protein corresponding to the 10 kD immunopositive region. We hypothesize that the developmental disappearance of 3D10 immunoreactivity relates to maturational events in specific monoaminergic circuits. Supported by grants HD20327, TOF/SAT, NS24386-JEC and HD0147-MR.

405.8
PHENOCIAL COCAINE EXPOSURE INCREASES BRAIN GANGLIOSIDE AND NEUTRAL GLYCOLIPID CONTENT OF NEONATAL RATS. K.C. Leschler*, G.H. Jackson*, C.A. Moody*, and J.L. Spear*. SPON: K. Reid. Dept, Anat. Sci. & Neurobiol., Univ. of Louisville Sch. of Med., Louisville, KY 40292, and 2Dept. Psychol. and Centers for Develop., Psychobiol. and Neurobehav. Sci., SUNY-Binghamton, Binghamton, NY 13901. Recent studies have reported behavioral dysfunctions in offspring exposed in utero to cocaine, along with alterations in the NA system. However, few other biochemical studies have been conducted. Given their role in fiber extension and neuronal maturation, gangliosides and neutral glycolipids were examined. Cultured cortical neurons and cell lines that have been isolated, sequenced and their mRNAs and genes cloned by recombinant DNA techniques. A 43 amino acid polypeptide, termed thymosin beta 10, was found to be very abundant in the early rodent nervous system. Following gel electrophoresis and immunoblot procedures antibody 3D10 appears to react with a single high molecular weight protein corresponding to the 10 kD immunopositive region. We hypothesize that the developmental disappearance of 3D10 immunoreactivity relates to maturational events in specific monoaminergic circuits. Supported by grants HD20327, TOF/SAT, NS24386-JEC and HD0147-MR.

405.9
ANTIBODY 3D10 IMMUNOREACTIVITY IN MONOAMINERGIC T.O. Fox, E.K. Shriver Center, Depts. of Biochemistry and Developmental Neurobiology, Waltham, MA 02254 and ^Washington Univ., Div. Biology & Medicine, St. Louis, MO 63110. The 3D10 monoclonal antibody (Tobet & Fox, Dev. Brain Res., In press, 1989) recognizes an antigen(s) in developing monoaminergic cell groups. Significantly more 3D10 immunopositive cells are observed in perinatal compared to adult rats. The experiments were examined the neurotransmitter specificity of 3D10 immunopositive neurons, as well as the presence of 3D10 immunoreactive antigen(s) in neuronal terminal regions and the protein(s) recognized.

405.10
EFFECTS OF PROMETHAZINE HC1 ADMINISTRATION TO GRAVID RATS ON BRAIN AMINE LEVELS IN OFFSPRING. M.S. Brander* and J.H. Patton. Department of Psychology, Baylor University, Waco, TX 76798. Promethazine HCl (Phenergan®, Wyeth) is a phenothiazine widely used for its anti-emetic and sedative potential during human pregnancy. Previous research has suggested that neurologic administration to gravid rats results in neurochemical changes in their offspring (Rosengarten & Freidhoff, Science, 203: 1133, 1979). Sprague-Dawley rats were exposed to Promethazine HCl during the gestation and lactation stages of development. The drug was injected into dams subcutaneously at a dose of 3.0 mg/kg per day. Offspring's brains were examined at both 1 and 21 days of age. No differences were observed at any age. The finding of brain gangliosides have been observed. These data suggest that biochemical consequences of gestational exposure to cocaine, or its Ca™ chelating metabolite benzoylecgonine, may be far-reaching and not restricted merely to the DA system. Supported by DA04478 (L.P.S.) and NS21057 (K.C.L.).
PROTEIN EXPRESSION IN POSTNATAL HIPPOCAMPUS AS ANATOMY, UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL, PISCATAWAY, NJ 08854, + DEPT. OF BIOLOGICAL SCIENCES, RUTGERS UNIVERSITY, PISCATAWAY, NJ 08855, + DEPT. OF BICLICAL SCIENCES, NEW YORK UNIVERSITY, MEDEICAL SCHOOL, 1000 3RD AVENUE, NEW YORK, NY 10022.

Banding patterns characteristic of the younger ages (P0, P2, P5) were distinct from the banding patterns of the older mice. In the younger mice by polyacrylamide gel electrophoresis (SDS/PAGE) was performed using 12% one-dimensional gels, the approximate molecular weights of:

1. 52-57 kD bands (approx. 52-57 kD and 45-48 kD) which were absent prior to P5 appeared or became increasingly more visible in the older mice. The banding patterns is as yet unclear. They could represent changes in gene expression or changes in posttranslational modifications of proteins. Further fractionation of these complex protein samples followed by two-dimensional gel electrophoresis may more definitively demonstrate the role in the developmental emergence of sensitzation. As a first step in this analysis, we have begun to examine cytoate activity and its modulation by SHT at different stages of juvenile development.

Cytocase activity was assessed in whole-CNS homogenates by examining the conversion of [6,6-MAP] into [AMP. To map the enrichment, we first examined adult animals. Constitutive cytocase activity was detected (x specific activity = 40 ± 5 pmol/min-mg protein, p<.001, N=12 [6 homogenates]), and was significantly increased by SHT (x increase over basal stimulation = .66%, p<.001, N=7 [6 homogenates]). We next examined Early Stage 12 juveniles. Here also, there was significant basal activity of the cytase (x specific activity = 54 ± 2 pmol/min-mg protein, p<.001, N=8 [2 homogenates]), which was also enhanced by SHT (x increase = .66%, p<.02, N=4 [2 homogenates]). Preliminary experiments show comparable results in Late Stage 12 juveniles.

Our results show that in Early Stage 12 adenylate cycase is present in the CNS of Aplysia and can be modulated by SHT. Thus far we have only studied the effect of SHT on whole-CNS homogenates. We are currently examining:
1. (1) other transmitters (e.g. SOFA), and (2) homogenous populations of neurons (e.g. sensory neuron clusters), to more quantitatively assess the role of adenylate cycase and its modulation in the development of sensitzation.

WHAT IS THE RELATIONSHIP BETWEEN THE DEVELOPMENT OF CHOLINERGIC SPINAL NEURONS IN MARINE SLICE CULTURES AND TOTAL CELL PROTEIN PRODUCTION?

M.S. Fisherman and M.L. Stewart. Dept. of Biology, Texas Woman's University, Denton Texas 76204.

The goal of our research is to establish in an in vitro model system current under investigation is a slice culture consisting of 200-300 um transverse sections of fetal mouse lumbar spinal tissue. In order to determine the suitability of this preparation for future electrophysiological studies, we have characterized both the cholinergic neurons and the total cell protein production in the cultures at different ages.

Cultures were stained for identification of cholinergic neurons at day 0 (day of culturing), three weeks, and five weeks in culture. At corresponding intervals, cultures were incubated in medium containing 35-S-methionine and examined for 1) precipitable counts via scintillation counting, and 2) electrophoresis protein patterns. Preliminary data suggest that changes in size and density of cholinergic neurons at the sampled intervals in culture is clearly reflected in total cell protein profile. (WMIB29 NS 22250-01)


Analysis of whole hippocampal tissue from C57B1/6j or BALB/cByj mice by polyacrylamide gel electrophoresis (SDS/PAGE) was performed on postnatal days: 0, 2, 5, 14, 38, 114. Using 12% one-dimensional gels, the banding pattern from the day and which develop during the period of the older mice. In the younger samples (P0, P2, P5) were distinct from the banding patterns of the older mice. In the younger samples (P0, P2, P5), six distinct bands were identified which were clearly distinguishable in the older mice. The bands had approximate molecular weights of: 1) 200-225 kD, 2) 97-100 kD, 3) 62-68 kD, 4) 52-57 kD, 5) 45-48 kD and 6) 28-31 kD. In addition, two bands (approx. 45-48 kD and 45-48 kD) which are absent prior to P5 appeared or became increasingly more visible in the older mice. The differences between the two age groups were clear using silver stained gels but some of the differences can also be detected with Coomassie blue staining. The development of these differences in banding patterns is as yet unclear. They could represent changes in gene expression or changes in posttranslational modifications of proteins. Further fractionation of these complex protein samples followed by two-dimensional gel electrophoresis may more definitively demonstrate specific changes in protein composition during hippocampal development.


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DEPOLARIZATION INDUCED CHANGES IN SYNAPTIC VESICLE PROTEIN (PS6) EXPRESSION IN SSG EXPLANTS. K.M. Lindemans* and K.F. Greif. Bryn Mawr College, Bryn Mawr, PA 19010.

PS6 is an integral membrane protein of synaptic vesicles which appears in synaptic vesicles of various transmitter types. We have shown PS6 to be regulated transsynaptically in the superior cervical ganglion (SCG) of adult and neonatal rats (Greif and Tremend, 1988). Pharmacological studies suggest that impulse activity is at least partially responsible for this transsynaptically regulated in vivo (Greif, J. Neurosci. 8:6268, 1988).

In vitro studies in defined medium support the role of depolarization in the regulation of PS6 levels. Neonatal SCG explants treated with the sodium channel ionophore, veratridine, or elevated potassium express 60% more PS6 than controls. This effect is reversible by tetraodoxin treatment. PS6 was metabolically labelled in neonatal SCG explants to determine the role of new protein synthesis in the increases of PS6 caused by depolarization. SCG explants were treated with veratridine for 36 hours and then pulse-labelled with 3H-histidine for two hours in the presence or absence of cycloheximide, a protein synthesis inhibitor. PS6 was purified by monoclonal antibody affinity column. Elutions were counted for two minutes on a stimulation counter. Veratridine treatment significantly increases (4-6p5 over control levels by 64% (SEM = 7, P < 0.005, t-test). Concurrent cycloheximide treatment with veratridine lowers 3H-PS6 levels by 40-60% KC1. The neurite loss induced by 3H of veratridine was reversible, so that a partial recovery has been observed after veratridine removal. Blockers of voltage-sensitive Ca++ channels; at 5M there was a loss in AChE-containing neurites whereas a higher concentration of veratridine led also to a marked reduction in the number of neurites in control explants. These effects could be elicited also by 40-60 m KC1, the neurite loss induced by 3H of veratridine was reversible, so that a partial recovery has been observed after veratridine removal. Blockers of voltage-sensitive Ca++ channels - verapamil and nifedipine - did not alter the effect of veratridine on neurite morphology. These observations and the finding that astrogial cells increased the basal number of AChE-positive cells in serum-free cultures indicate that the neuronal activation state and the interaction with glial cells affect the survival and expression of AChE-positive neurons. Supported by Dysautonomia Foundation, Inc. and NSF grant BNS 85-19873 to KFG.
ANATOMY AND PHARMACOLOGY AND THERAPEUTICS, AND DIV. OF ARBORIZATION OF DEVELOPING CEREBELLAR PURKINJE CELLS.

between each new generation of branches. New growth

LOW DOSE HYDROGEN SULFIDE AND ITS EFFECTS ON THE DENDRITIC

pmm or 50 pmm H 2S for 7 hours per day in an environment

postnatal, representative pups from each litter were

Controls were similarly treated including placement in an

the dendritic arborization. (Supported by Alberta

in vertex path length suggesting an increased distance

the development of membrane excitability in embryonic rat spinal cord cells was examined using computerized digital imaging of fluorescent voltage-sensitive dye signals. The system included an epifluorescence microscope, image intensifier, and digitalizing frame grabber. Four independent images were averaged for each measurement and allowed to analyze a continuous recording of the entire field of cells under baseline conditions and in response to successively applied electrolytes or ligands.

Rat spinal cord was harvested on embryonic days 13, 15 and 17 (E13, E15, E17) and enzymatically dissociated into single cell suspensions, placed in culture dishes and allowed to adhere for 2 hours. Culture dishes were perfused with continuously flowing solutions containing 100 nM of the voltage sensitive dye DiIC 2(5). These cells all showed reproducible and reversible changes in fluorescence indicating membrane depolarization in response to increases in extracellular potassium concentration. Veratridine was used as a probe for the voltage sensitive sodium channel and depolarizing responses to veratridine were largely absent from cells dissociated at E13 but developed progressively so that by E17, almost all cells responded. This response was blocked by tetrodotoxin or use of sodium-free medium.

This preliminary data suggests that the sodium-selective voltage-sensitive channel activated by veratridine develops subsequent to E13 in the rat spinal cord, in agreement with previous reports using flow cytometry (Rothberg et al., 1986). Further study of this technique will be useful for investigations of cultured cells as it allows for simultaneously recording multiple cells while at the same time monitoring each individual cell's response to successively applied conditions.

ANALYSIS OF CALCIUM FLUXES IN DISSOCIATED RAT NEURONS

DURING EMBRYONIC DEVELOPMENT. S. M. O'Connell*, J. P. Grierson.

Embryonic rat brain can be investigated using the technique of flow cytometry and the fluorescent probe Indo-1. This approach obviates the possibility of premature differentiation when embryonic cells are placed into tissue culture. Freshly isolated cells were incubated in Indo-1 AM for 30 minutes followed by a rinse and a 30 minute post-incubation period. The efficiency of Indo-1 hydrolysis was tested for each experiment by permeabilizing both cell types with triton X-100. Analysis of dissociated cell preparations from the E14-E17 hypothalamus reveals that about 25 to 30% of cells respond to a stimulus of 25 or 50 mM KCl. The significant influx of Ca 2+ into cells obtained from the cerebral cortex are responsive under the same conditions and from the same embryonic ages. The Ca 2+ influx in dissociated cells from both regions is markedly attenuated by 1 µM nifedipine, however only the cortical cells appear to be potentiating by 1 µM Bay K. The responding cells from both regions have been shown to be neurons by sorting on the basis of [Ca 2+]i, maintaining in culture and then staining for MAP2 immunoreactivity. Furthermore, the detection of K-stimulated Ca 2+ uptake by astrocytes is believed to be negligible, since experiments with cultured astrocytes indicate a very low level of Indo-1 hydrolysis by these cells. It can be inferred that cells from the embryonic brain preparations also express voltage-sensitive sodium channels (VSSC) since the addition of tetrodotoxin attenuates the Ca 2+ response when compared with KO alone, suggesting that in the control situation depolarization is augmented by Na 2+ influx through VSSC. Supported by NIH NS 25168.

N E U R O T Y P O X I T O X I CIT Y: ME T A L S A N D O R G A N I C S

LOW DOSE HYDROGEN SULFIDE AND ITS EFFECTS ON THE DENDRITIC ARBORIZATION OF DEVELOPING CEREBELLAR PURKINJE CELLS.

K.R. Hanack*, R. Benitez*, and S.H. Roth. Deps. of Anatomy and of Pharmacology and Therapeutics, and Div. of Toxicology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Hydrogen sulfide (H 2S) is an environmental pollutant which can produce severe effects on the adult CNS; however, little is known about its effects on developing CNS. Pregnant rats (Sprague–Dawley) were exposed to either 20 ppm or 50 ppm H 2S for 7 hours per day in an environment chamber from gestation day 15 until postnatal day 14. Controls were similarly treated including placement in an environmental chamber flushed with room air. On day 21 postnatal, rats were perfused, immersion fixed and immunocytochemicals of the cerebellum and processed for Golgi staining. Ten complete Purkinje cells were selected from each group and dissected to determine the receptive field, soma size, and position of dendritic arborization. (Supported by Alberta Occupational Health and Safety Heritage Grant Program).


We examined the development of spontaneous firing in dissociated hippocampal neurons using a loose patch recording method. Hippocampal neurons were dissected from E17 rat brains and plated onto a confluent monolayer of the mature cortical astrocytes. Extracellular recordings were made from neurons, which are 10-25 µM diameter, with whole cell electrodes beginning 20 µm from the cell membrane. The resting membrane potential ranged from -50 to -80 mV and the input resistance ranged from 15 to 50 MΩ. Voltage clamp recordings were obtained from the cerebral cortex and the hippocampus and could be achieved by 150 µM and 600 µM tetrodotoxin, respectively. The data showed that the dendritic arborization is markedly attenuated by 1 µM nifedipine, however only the cortical cells appear to be potentiating by 1 µM Bay K. The responding cells from both regions have been shown to be neurons by sorting on the basis of [Ca 2+]i, maintaining in culture and then staining for MAP2 immunoreactivity. Furthermore, the detection of K-stimulated Ca 2+ uptake by astrocytes is believed to be negligible, since experiments with cultured astrocytes indicate a very low level of Indo-1 hydrolysis by these cells. It can be inferred that cells from the embryonic brain preparations also express voltage-sensitive sodium channels (VSSC) since the addition of tetrodotoxin attenuates the Ca 2+ response when compared with KO alone, suggesting that in the control situation depolarization is augmented by Na 2+ influx through VSSC. Supported by NIH NS 25168.


NEUROTOXICITY: ME T A L S A N D O R G A N I C S

LOW DOSE HYDROGEN SULFIDE AND ITS EFFECTS ON THE DENDRITIC ARBORIZATION OF DEVELOPING CEREBELLAR PURKINJE CELLS. K.R. Hanack*, R. Benitez*, and S.H. Roth. Deps. of Anatomy and of Pharmacology and Therapeutics, and Div. of Toxicology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Hydrogen sulfide (H 2S) is an environmental pollutant which can produce severe effects on the adult CNS; however, little is known about its effects on developing CNS. Pregnant rats (Sprague–Dawley) were exposed to either 20 ppm or 50 ppm H 2S for 7 hours per day in an environment chamber from gestation day 15 until postnatal day 14. Controls were similarly treated including placement in an environmental chamber flushed with room air. On day 21 postnatal, rats were perfused, immersion fixed and processed for Golgi staining. Ten complete Purkinje cells were selected from each group and dissected to determine the receptive field, soma size, and position of dendritic arborization. (Supported by Alberta Occupational Health and Safety Heritage Grant Program).


Postnatal lead (Pb) exposure alters the development of the hippocampus, a brain region critical in memory function. Because Pb is sequestered in this region, others have proposed that the manifestations of Pb toxicity may be caused by a Pb-induced reduction of hippocampal Zn. To test this hypothesis, we have measured Pb and Zn in microdissected hippocampal subfields after postnatal Pb exposure. Lactating dams ingested water containing either 0.2% or 2.5% Pb for 8 weeks in utro. 45 of 46 Pb-exposed pups were sacrificed and Pb and Zn concentrations determined in each subfield by atomic absorption spectrophotometry. Dose-related decreases were observed in Pb-exposed animals compared to controls, the concentration of Zn in the whole hippocampus was not perturbed. Moreover the concentration of Pb in the subicular field was altered by Pb exposure during postnatal development. Although Zn and Pb are both enriched in the hippocampus, these data indicate that they probably do not compete for binding to the same ligand. These results complement the study of Petit and Le Boutillier (Neurotox 7:237, 1986) which demonstrated behavioral disabilities in postnatal Zn deficiency and Pb toxicity.
407.3
NEUROCHEMICAL EFFECTS OF PRENATAL LEAD EXPOSURE IN GUINEA PIGS. T.K. Bowles*, W.D. Baker, and E. Tiffany-Castiglioni*. Va/Md Regional College of Veterinary Medicine, Blacksburg, VA 24061.

Blood lead (Pb) levels correlate with cognitive dysfunction in children, and umbilical cord blood levels indicate many children are exposed to Pb levels above the significant Pb levels. Since the effects of such exposure are still unclear, we are using the guinea pig as an animal model of prenatal exposure to further study this problem. Guinea pigs were exposed to one of 3 levels of Pb daily during the last 2 trimesters of pregnancy. On postnatal day 38, their offspring were killed by head-focused microwave irradiation and specific brain regions collected for analysis of neurotransmitter levels by HPLC-ED. Lead exposure resulted in no changes in maternal weight gain, litter size, birth weight, or neonatal growth rates. However, lead exposure did result in significant increases in metabolic levels of dopamine and serotonin in several brain regions. HVA levels increased with Pb treatment in frontal cortex, striatum, septum, thalamus, and inferior colliculus, but DOMA levels did not change. Dopamine levels decreased only in the inferior colliculus. 5-HIAA levels also showed widespread increases, but serotonin levels did not change. There appears to be an increased metabolism of these neurotransmitters in animals exposed prenatally to Pb. Funded by BRSG, Virginia Tech.

407.4
INDUCTION OF NEURONFIBRILLARY DEGENERATION IN FETAL RABBIT MOTOR NEURON-ENRICHED CULTURES. R.M. Garruto and M.J. Strong*. LCNSS, NINDS, NIH, Bethesda, Maryland, 20892.

A consistent feature of the aluminum-induced encephalomyelopathy of rabbits is the presence of neurofibrillary inclusions in motor neurons. To investigate the mechanisms underlying this phenomenon, we exposed fetal rabbit motor neuron-enriched cultures to varying concentrations of aluminum chloride. At 16 days in culture, when all major neurofilament subunit proteins are present, the media was supplemented with either 10 mM, 1 mM or 100 mM aluminum chloride. Within 48 hours, the cultures exposed to 10 mM and 1 mM aluminum chloride developed refractile intracytoplasmic inclusions and rare focal neuritic swellings. By 96 hours these features were present in all cultures, with motor neurons exposed to 10 mM aluminum chloride developing signs of cell death. The inclusions demonstrated immunoreactivity with monoclonal antibodies against phosphorylated neurofilament. These observations demonstrate that dissociated fetal rabbit motor neuron in vitro recapitulate the aluminum-induced neurofibrillary inclinations of rabbit motor neurons in vivo, thereby providing a dynamic model of aberrant neurofibrillary degeneration in nontransformed cells. (*Supported in part by a research fellowship from the Medical Research Council of Canada.

407.5

Polychlorinated biphenyl (PCB) is an ubiquitous environmental pollutant, the ingestion of which functions to the regulation of many important neurochemical processes. Recent studies in our lab have shown PCB feeding to pregnant rats results in widespread decreases in the activity of choline acetyltransferase (ChAT) in the hippocampus and basal forebrain of 15 day old pups. Since hypothyroidism has been shown to depress ChAT activity in young rats it was of interest to determine whether PCB administered through the mother, would have a similar effect. Female rats were fed PCB (Aroclor 1254) at 0.25% of a standard diet throughout pregnancy and lactation. Fifteen day old pups were decapitated, and the activity of ChAT in hippocampus and basal forebrain was estimated by the ability of an homogenate to incorporate [14C]-labeled acetyl-CoA into acetylcholine. When activity was expressed as nmol product generated/mg soluble protein/hr, PCB depressed hippocampal ChAT to about 60% of normal and that in basal forebrain to 40%. Thus it appears that PCB has undesirable effects on brain neurotransmitter systems. This may result from concomitant hypothyroidism or direct PCB effects. (Supported by BRSG #89-02).

407.6

Exposure of adult rats to polychlorinated biphenyls (PCBs) decreases regional brain biogenic amine function (Seegal et al., BBA, 1, 1 Part 2, p. 883, 1988). To determine the functional significance of these changes we have examined the effects of PCBs and catecholaminergic drugs on locomotor activity.

We exposed adult rats to control chow or chow adulterated with 500 or 1000 ppm Aroclor 1254 for sixty days. Locomotor activity was determined every third day in infra-red photocell activity cages. Beginning on about post-exposure day 30 animals were injected s.c. with either saline, 0.25, 0.5 or 1.0 mg/kg d-amphetamine sulfate thirty minutes before testing.

Spontaneous activity in the PCB-exposed animals was significantly lower than in control animals. D-amphetamine significantly increased activity although PCBs attenuated the drug-induced increase in locomotor activity. Thus, PCB exposure of the adult rat decreases both spontaneous and drug-induced locomotor activity. These effects on locomotor activity may be causally related to concomitant decreases in central catecholamines.

407.7

Capsaicin, a neurotoxin known for its ability to selectively damage small unmyelinated primary sensory neurons, is also capable of producing degeneration at certain highly specific sites along the entire neuroaxis in rats. In this experiment, we examined capsaicin-induced CNS degeneration in rats of different ages. Rats were anesthetized, injected systemically with a single high dose of capsaicin (75-100 mg/kg) or vehicle solution at 10, 15, 20, 25, or 30 days or 3 months of age and sacrificed at times optimal for observing capsaicin-induced degeneration (6-18 hr post injection). Brains and spinal cords were prepared and stained with cupric-silver. At sites where degeneration was present in all age groups, the staining was generally more intense in the 10-30 day old animals than in adults. Several areas of degeneration observed in 10-30 day old rats were not observed in adults: portions of the bed nucleus of the stria terminalis, the septohypothalamic nucleus, medial preoptic area, ventral reuniens nucleus, ventromedial hypothalamus, lateral habenula and spenoid nucleus. In the ventrolateral geniculate and olivary pretectal nucleus, staining was intense in pups, but very light staining was sometimes also seen in adults. The intensity and location of staining was similar between 10 and 30 days except in the habenula and spenoid nucleus where degeneration declined progressively between 10 and 30 days of age. Results suggest that the sensitivity of some CNS projections to capsaicin's toxicity is age-related. Factors contributing to this change in sensitivity are not known.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
408.1 THE EFFECT OF CHRONIC ETHANOL CONSUMPTION ON CELL NUMBER IN THE HOUSE CEREBELLAR CORTEX. R.M. Napper* and B.H. Harvey, Dept. Anatomy, Neuroscience Centre, Univ. of Otago, Dunedin, New Zealand.

In the present study, quantitative methods have been used to determine the number of cells in larval and postnatal brains for rats which had been exposed to ethanol in utero. Rats were exposed to ethanol (40 or 120 mg/kg per day) or to water for 15 days during gestation. After weaning, the rats were exposed to ethanol (40 or 120 mg/kg per day) or to water for 45 days. The results show that ethanol consumption during gestation and lactation results in a decrease in cell numbers in the cerebellum. This decrease is more pronounced in the anterior part of the cerebellum than in the posterior part.

408.2 A MODEL TO STUDY THE EFFECTS OF CHRONICALLY INFUSED NEUROPHARMACOLOGICAL AGENTS ON DEVELOPING EMBRYOS. B. E. Bell, T. A. Jones, UNC College of Medicine, Lincoln, NC 27695; and H. W. Morley, Boyceton, NY 14821.

The purpose of this investigation was to develop an experimental model that could be used to assess the effects of neuropharmacological agents on developing embryos in situ. A method of chronic in vivo drug infusion was developed using a percutaneous cannula (Tew, 1984). Neurophysiological effects of drug administration were assessed by recording compound action potentials of the vestibular nerve (Jones & Tew, 1984). Nicotine levels in serum were estimated for the present study. Animals were divided into four groups: 1) control, 2) sham (vehicle only), 3) continuous drug and 4) drug followed by vehicle only. Cannulas were implanted in 18 eggs and sealed in place under sterile conditions to enable continuous infusion of drug and vehicle (i.e., 6.03 mg/ml over 10 days). The resulting nicotine levels in serum (S) and extra-embryonic fluid (EEF) were determined (SIR: 21.4 ± 8.3 mg/ml, EEF: 97.0 ± 8.9 mg/ml). On the nineteenth day of development, the eggs were opened to access embryos and subcutaneous skull electrodes were placed. Vestibular response thresholds and input/output functions were determined. The animals were then decapitated, brains removed and prepared for receptor binding studies. 1H-nicotine was used in binding assays to estimate brain nicotine acetylated receptor densities. The study demonstrates the usefulness of the avian model in research evaluating the consequences of exposing embryos to neuropharmacological agents.


In the present study, quantitative methods have been used to determine the number of cells in larval and postnatal brains for rats which had been exposed to ethanol in utero. Rats were exposed to ethanol (40 or 120 mg/kg per day) or to water for 15 days during gestation. After weaning, the rats were exposed to ethanol (40 or 120 mg/kg per day) or to water for 45 days. The results show that ethanol consumption during gestation and lactation results in a decrease in cell numbers in the cerebellum. This decrease is more pronounced in the anterior part of the cerebellum than in the posterior part.

408.4 SWIMMING BEHAVIOR IN MICE Sowered BY ALCOHOL CONSUMING PARENTS. P. J. Blijterke and E. L. Abel, Dept. of Ob/Gyn, Wayne State University School of Medicine, Detroit, MI 48202.

Male mice consumed liquid alcohol diets containing 25%, 10% or 0% ethanol derived calories (ECD). Animals receiving the 10 and 0% ECD diets were pair fed to those consuming the 25% ECD diet. After seven or fourteen weeks of consumption, males were bred to non-treated females. Offspring were tested for swimming behavior at 75 days of age. Offspring sired by alcohol-consuming males were more immobile than controls. Propranolol (1, 3 mg/kg) significantly increased immobility in all groups but did not reverse the effects of paternal alcohol consumption. Metoprolol (1 mg/kg) increased immobility in all groups but did not reverse the effects of paternal alcohol exposure. AMPH (100 mg/kg) did not affect swimming behavior. These results extend the evidence for paternally mediated behavioral mutagenesis. Supported by P50 AA07606 and AA 06999.

408.5 EFFECT OF PRENATAL EXPOSURE TO ETHANOL ON GENE EXPRESSION DURING RAT BRAIN DEVELOPMENT. D. Macekiewicz-Lenior and P. J. Miner, Research Institute of Scripps Clinic, La Jolla, CA 92037.

There is considerable evidence that prenatal exposure to ethanol results in abnormal development of the nervous system. To investigate this phenomenon at the molecular level, we have examined exposure to ethanol on the postnatal expression of particular brain genes. Pregnant rats were exposed to ethanol vapor between days 3-31 of gestation, resulting in blood alcohol levels between 150 and 200 mg%. At birth, pups from the ethanol-treated rats and from untreated control animals were fostered to non-mothers. Ethanol-treated and control pups were sacrificed at different times after birth and RNA was extracted from four dissected brain regions: hindbrain, cerebellum, midbrain and cortex. The expression patterns of particular mRNAs were analyzed on Northern blots and by solution hybridization. The mRNAs tested were chosen to represent genes expressed in different CNS cell types and included mRNAs encoding MAG,1B36, PLP, 1α-tubulin and GFAP. The results show differences in mRNA expression between control and ethanol-exposed animals with some variation among brain regions. In general, the data indicate that ethanol causes a slowing or delay in the normal time course of expression of the marker genes during brain development and may alter levels of expression. Supported in part by NIAAA Alcohol Research Center grant AA06420.

408.6 DEFICITS IN RADIAL MAZE LEARNING IN ADULT RATS FOLLOWING ALCOHOL EXPOSURE DURING THE BRAIN GROWTH SPURT: ASSOCIATION OF WORKING MEMORY IMPAIRMENTS WITH CA1 NEURON LOSS. C.B. Goodlett, D.J. Brownstone, P.A. Wasserman* and J.B. West, Univ. of Iowa, Iowa City, IA 52242.

The most serious aspects of fetal alcohol syndrome (FAS) in humans are long-term deficits in cognitive function. Adequate models of permanent cognitive deficits in animals produced by alcohol exposure during development have been difficult to develop. We report here that alcohol exposure to neonatal rats during the brain growth spurt, comparable to third trimester in humans, results in severe learning deficits in a radial maze task, including working memory deficits correlated with hippocampal cell loss in CA1. During postnatal days 4-9, rat pups were exposed to ethanol (10%) or vehicle (+10) of alcohol per day using artificial rearing procedures, which resulted in peak blood alcohol concentrations of 415 and 335 mg/dl, respectively. Control groups included artificially reared pregnant females and normally reared suckle controls (n=18). As adults, they were tested for 30 days on a 12-arm radial maze, in which six arms were consistently baited and six were not. The alcohol-treated rats committed significantly more errors related to working memory (within-session repeated arm entries), and also more errors related to reference memory (initial entries into unbaited arms). However, the working memory deficits were much more pronounced in animals that were exposed to ethanol during the brain growth spurt. These results provide further support for the hypothesis that FAS is associated with CA1 cell counts, suggesting that in this model of FAS, deficient spatial information processing is associated with CA1 hippocampal cell loss. (Supported by NIAAA grants #AA05523 and AA07313).
408.7
ALCOHOL EXPOSURE DURING THE BRAIN GROWTH SPURT INDUCES PERMANENT MICROENCEPHALY AND NEURAL DEFORMITIES IN RATS. J.R. West, D.J. Bonthius and C.R. Goodlett. (SPON: J.D. Coulier). Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

A rat model of alcohol-related developmental effects was used to examine the long-term morphological changes associated with developmental alcohol exposure. Sprague-Dawley rat pups were reared artificially over postnatal days (PD) 4-12 (a period of rapid brain growth similar to the human third trimester). On PD 4-9, alcohol-exposed groups received either 0.6 or 7.5 g/kg/day ethanol administered as 7.5% and 8.5% v/v solutions, respectively, for 4 of the 12 daily feedings. Controls included artificially reared (gastrostomy) and normally reared suckle controls. Pups were fostered back to dams on PD 12, weighed on PD 21 and perfused on PD 100. Brain weights were measured. Cerebellar Purkinje cells in all 10 vermal lobules and hippocampal neurons in fields CA1, CA2/3 and CA4 were counted from single sections, the following cell populations were counted: hippocampal neurons in fields CA1, CA2/3 and CA4, dentate gyrus granule cells, cerebellar Purkinje cells and olfactory bulb mitral cells. Cerebellar granule cells were estimated. Neuronal populations were not affected equally by the alcohol treatments. In the hippocampus, CA1 pyramidal cells were significantly reduced by both alcohol treatments, while CA2/3 and CA4 were not reduced by either treatment. There was a significant loss of Purkinje cells in the cerebellum, and some lobules were significantly more affected than others. The lobules in which Purkinje cells were most mature at the time of alcohol exposure (lobules I, II, IX and X) were the most vulnerable to Purkinje cell loss. Therefore, developmental alcohol exposure induces permanent neuronal loss which is population- and region-specific. (Supported by NIAAA grant AA05523.)

408.8
A SINGLE DAY OF ALCOHOL EXPOSURE IN NEONATAL RATS INDUCES CEREBELLAR PURKINJE CELL LOSS. B.L. Marcussen*, C.R. Goodlett and J.R. West. Univ. of Iowa, Dept. of Anat, Iowa City, IA 52242.

The cerebellum in the neonatal rat is highly susceptible to ethanol-induced Purkinje cell death following exposure on postnatal days (PD) 4-10. We examined whether ethanol exposure on a single day during the brain growth spurt could induce Purkinje cell loss. Rat pups were assigned to one of four groups on PD 4: 6.6 g/kg/day ethanol group, 3.3 g/kg/day ethanol group, gastrostomy and suckle control groups. The ethanol and gastrostomy control groups were reared artificially. Ethanol-exposed groups were given two consecutive ethanol-containing feedings on PD 4. All subsequent feedings were with milk formula alone. The rats given 6.6 g/kg ethanol had high peak blood ethanol concentrations (mean = 352 mg/dl). On PD 10 all rats were perfused, the brains extracted, postfixed and the cerebellar vermis isolated and embedded in JB-4. Purkinje cells were counted from 2 mm thick midsagittal sections stained with cresyl violet/safronin O. Suckle and gastrostomy controls did not differ significantly in the overall number of Purkinje cells. The rats given 6.6 g/kg ethanol had significant reductions in Purkinje cells compared to all other groups (p<0.01). Additional analysis revealed significant reductions in lobules 1-5 and 9-10 but not in lobules 6-8. The pups exposed to 3.3 g/kg ethanol demonstrated significant reductions in overall number of Purkinje cells compared to suckle controls but not compared to gastrostomy controls. The Purkinje cell loss in this study implies that a brief exposure to a relatively high blood alcohol level during the brain growth spurt, as may occur in 'binge' drinking during the third trimester, may constitute an increased risk to vulnerable cell populations in the developing central nervous system. Supported by grant AA05523 to JRW.

408.9
ALCOHOL-INDUCED NEURONAL LOSS IN DEVELOPING RATS: DIFFERENCES IN VULNERABILITY AMONG CELL POPULATIONS. D.J. Bonthius, C.R. Goodlett and J.R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

A rat model of third trimester alcohol administration was used to examine differences in vulnerability to alcohol-induced cell loss among neuronal populations. Sprague-Dawley rat pups were reared artificially over postnatal days 4-10 (a period of rapid brain growth similar to the human third trimester). Alcohol-exposed pups received 4.5 g/kg/day ethanol in either 2 or 4 of the 12 daily feedings (administered as 5% v/v solutions, respectively). Controls included non-alcohol exposed gastrostomy controls and normally reared suckle controls. On postnatal day 10, the pups were perfused with fixative, and the brains were removed. Two-micron-thick sections were cut horizontally through the midtemporal hippocampal formation, sagittally through the midline cerebellar vermis and coronally through the olfactory bulb. From single sections, the following cell populations were counted: hippocampal neurons in fields CA1, CA2/3 and CA4, dentate gyrus granule cells, cerebellar Purkinje cells and olfactory bulb mitral cells. Cerebellar granule cells were estimated. Neuronal populations were not affected equally by the alcohol treatments. In the hippocampus, CA1 pyramidal cells were significantly reduced by both alcohol treatments, while CA2/3 and CA4 were not reduced by either treatment. There was a significant loss of Purkinje cells in the cerebellum, and some lobules were significantly more affected than others. The lobules in which Purkinje cells were most mature at the time of alcohol exposure (lobules I, II, IX and X) were the most vulnerable to Purkinje cell loss. Therefore, developmental alcohol exposure induces permanent neuronal loss which is population- and region-specific. (Supported by NIAAA grant AA05523.)

408.10

Maternal ethanol (ETOH) consumption during gestation has been demonstrated to induce alterations in the nervous system of the offspring. In this study the effects of maternal ETOH consumption on the neonatal spinal cord were examined. Six female Sprague-Dawley rats were placed on either an ETOH-containing liquid diet or an isocaloric ETOH-free liquid diet for 5 weeks, then mated. The diets were continued throughout gestation. On postnatal day 1, the litters were weighed and a mean weight/group determined. Four pups from each group whose weights were closest to the means were perfused, their C3 spinal cords removed, embedded and transverse thick sections of the entire cord obtained. Areal measurements were obtained using a computerized bit pad and the numbers of myelinated axons in the ventral funiculus (VF) assessed. While the mean weight of the pups exposed to ETOH was significantly reduced from the control group (p<0.01), no difference in the area of the C3 spinal cord or in the area of the gray matter was observed. However, the number of myelinated axons in the VF was significantly reduced (p<0.01) in the ETOH exposed pups. These results indicate that ETOH consumption prior to and during gestation does not effect the gross morphology of the spinal cord of the neonate, however, alterations at the axonal level are apparent.

408.11

Fetal alcohol exposure produces permanent laminar and cellular defects in the cerebellum. These malformations have been attributed to alterations in early developmental processes such as neuronal migration and differentiation. The present study examined the effect of gestational ethanol exposure on the laminar distribution of callosal projection neurons in mature rat neocortex. The subjects, 3-6 month old hooded rats, were the progeny of mothers prenatally fed a liquid diet containing 6.7% (v/v) ethanol, an isocaloric liquid control diet, or a chow diet. Horseradish peroxidase (HRP) was injected unilaterally into area 3 of primary somatosensory cortex. After a post-injection survival period of 2 days, each brain was processed for HRP histochemistry and stained with cresyl violet to identify laminar patterns. In control animals, callosal projection neurons were distributed in layers II-VI of the contralateral somatosensory cortex, but most HRP-positive neurons were in layers II/III and V of somatosensory cortex. In experimental animals, about 75% of the callosal projection neurons in ethanol exposed animals were in layers V and VI. We hypothesize that gestational ethanol exposure produces an abnormous distribution of callosal projection neurons in somatosensory cortex from the ectopic placement of cortical neurons or the abnormal differentiation of infragranular cortical neurons. Funded by AA 06916, AA 07568, DE 07734 and NS 07229.
MICRODIALYSIS, IN THE CISTERNA MAGNA, OF Na TRANSPORT INTO THE CEREBROSPINAL FLUID (CSF) SYSTEM. C.E. Johanson, G. Knoekey and A. Fowler. Program in Neurosurgery, Dept. of Clinical Neurosciences, Brown Univ./R.I. Hospital, Providence, RI 02902.

Microdialysis in vivo has been used to study extracellular compounds of CSF. This report describes an in vivo microdialysis approach and applies it to study the CSF dynamics in rats. The choroid plexus is the major source of CSF, which is produced by active transport of Na+ from blood to ventricles. By injecting Na-22 into cerebral circulation and measuring its movement into CSF by microdialysis, one should observe a measurable Na-22 signal from the choroid plexus of the brain.

A 200-300 g, anesthetized with ketamine and xylazine, were kept at 37°C while being monitored and blood gases and pH were measured at 0, 45 min intervals. Plasma turnover of Na-22 into the dialysate was reduced 45% by acetazolamide (25 mg/kg). Cysternal dialysate was measured nearly 30 min after the injection of Na-22 into the cisterna magna. It has long been thought that Mg does not pass the blood-brain barrier (BBB) but rather it's actions are all peripheral. Nervous system responses to Mg have been used as an example of the efficacy of Mg homeostasis in cerebral extracellular fluids. Further, it has been thought that by birth the BBB is intact for Mg. We report these results in light of observations made in developing swine to increasing plasma levels of Mg (Gootman, et al. Neurourol. 12, 1988) and the muscarinic agonist carbamol (CA). Cerbral spinal fluid (CSF) levels of Mg during IV infusion of MgCl2 in developing swine. L.T. Rivera, R.H. Lim, P.M. Gootman, H.L., Cohen, H. Gootman. Div. Pediatric. Cardiol. Schneider Children's Hosp., Long Is., A. Einstein Coll. Med., New Hyde Park, NY, 11042 and Dept. Physiol. SUNY-Hlth. Sci. Ctr. Bklyn., Bklyn, NY 11203.

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409.7

The growth potential in vitro of primary EC from different organs in 10 d iah/c mice was studied. Rapid purification of vascular EC from cerebral cortex (CTX), subcortical cerebral (CC), spinal cord (SC), cerebellum (CBL), kidney (KID), lung (LIN), liver (LIV) and spleen (SPL) used an enzyme digestion, Dextran-Percol step gradient technique. Initial cell collection was prepared at step gradients. Tissues were homogenized in 0.5% collagenase - 0.2% DNase. The first gradient through 15% dextran yielded viable cells at the top vascular space contaminated by rbc and wbc in the pellet. The pellet is resuspended for a second gradient in 45% Percol. A monodisperse EC band just touches the upper margin of a univ of Wisconsin Med Sch and Wm S Middleton VA Hosp,

purification of vascular EC from cerebral cortex [CΓX],

GRĂΛŒE1ΠS.

different from brain and non-brain organs. CΓX, sCΓX,
graber* and B.R. Brooks. ( S P C f t ſ : R.Daly) Neurology Dept,

ĮHDCHHEΓJAL CELLS [EC]

brain EC. The growth rate of CTX and CBL EC is faster
gelatin while fibronectin is essential for A, IDN, LIV

and SPL EC. Cultured EC are von Willėbrand Antigen

CBL, and KID EC grow out equally well on fibronectin and

subcortex [sCΓX], cerebellum [CBL], spinal cord [SC],

that their receptors exist in brain microvessels, for

their effect on cyclic GMP generation in isolated rat

brain microvessels is mediated, at least in part, by cyclic

stimulating guanylate cyclase. This stimulation was dose

results indicate that ANP receptor stimulation in brain

samples', mostly peptides for which there is evidence

extract was assayed by radioimmunoassay. In all

perchloric acid and the content of cyclic GMP in the

coli EC. Although growth of cells were observed, they

did not stain for Factor VIII related antigen (FVIIIag).

PMN-CEC interaction may enhance the formation of kinins, LTB4 and 6K but not TXB2.

PMN CEC A23187 (pg/ml) (ng/ml)

- + 44 ± 12 36 ± 2 27 ± 4 -
+ + 47 ± 18 6 ± 1 24 ± 2 +
+ + 87 ± 17 44 ± 3 26 ± 3 +
+ + 110 ± 5+

Results indicate that PMN-ECG interaction may enhance

isochemical and ionic effects on cyclic GMP generation in rat cerebral

microvessels. We have used an in situ perfused cerebral microvessel preparation to study the regulation of cyclic GMP formation in vivo.

Data show that ANP receptor stimulation in brain microvessels increases cyclic GMP levels.

Our results indicate that ANP receptor stimulation in brain microvessels is mediated, at least in part, by cyclic GMP. Receptors for the other vasoactive substances that were tested, if they exist in rat cerebral microvessels, are not related to cyclic GMP.

409.9

A number of laboratories have found the culture of endothelial cells from adult rat brain microvessels (MV) difficult to achieve. We therefore proceeded to develop a procedure for the isolation and culture of small ECMF. We followed traditional methods for isolation of MV. The vessels were purified through a dextran/percol gradient and a nylon mesh was used to isolate the microvessel fraction. ECMF were plated on fibronectin or gelatin coated dishes. Although growth of cells were observed, they did not stain for Factor VIII related antigen (FVIIIag). However, when the ECMF were plated on tripel (basement membrane collagen), positive staining for VLLIEC, transferrin receptor and incorporation of low density lipoprotein was observed. Initial studies were undertaken to test the viability of this preparation. Prostacyclin (PGI2) was measured after 24 hrs of equilibration as 6-Keto PGI2. Kininogen activation was assayed by radioimmunoassay in the spent medium. Kininogen to kinin and formation of leukotriene B4 (LTB4), bradykinin B2 (TXB2) and 6-ko-FCP (6K).

Table shows concentrations of mediators in serum-free medium following incubation of confluent CEC (0.22 ng protein/well) with or without PMNs (10^3 cells/ml).

"-" indicates no change.

Table:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Kinin</th>
<th>LTB4</th>
<th>TXB2</th>
<th>6K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
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<tr>
<td>PMN</td>
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PMN-ECG interaction may enhance the formation of kinins, LTB4 and 6K but not TXB2.

409.10

ICM can undergo a "metabolic shock" indicated by low initial ATP levels and ATP/ADP ratios that recover on incubation in enriched medium. To determine which fuels best maintain microvessel energy state, we incubated ICMV with single metabolic fuels and measured their ATP and ADP contents hourly using a bio-luminescent assay. Below are mean + SE results of three independent experiments.

<table>
<thead>
<tr>
<th>Exp. Conditions</th>
<th>Kinin</th>
<th>LTB4</th>
<th>TXB2</th>
<th>6K</th>
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<tbody>
<tr>
<td>Control</td>
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<td>PMN</td>
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Vascular dilatation is mediated by a series of mediators such as histamine, bradykinin, thrombin and ionomycin which induce a significant increase of PGI2 production (104%, 223% and 88% respectively). It appears that this preparation will be useful for further studies on the effect of a variety of substances, mostly peptides for which there is evidence that their receptors exist in brain microvessels, for their effect on cyclic GMP generation in isolated rat brain microvessels. Bulk tests of isolated rat cerebral microvessels were incubated with atrial natriuretic peptide (ANP), 10^-10 to 10^-7 M; angiotensin II, 10^-10 to 10^-7 M; bradykinin, 10^-10 M; carbachol, 10^-10 M; and thrombin, 10^-10 M for a period of 10 min after which the reaction was stopped and the microvessels were extracted with perchloric acid and the content of cyclic GMP in the extract was assayed by radioimmunossay. In all experiments, the ability of sodium nitroprusside (10^-4 M) to increase guanylate cyclase activity was ascertained. Of all the agents studied, only ANP was potent in stimulating guanylate cyclase. This stimulation was dose dependent, with maximum stimulation at 10^-6 M. Our results indicate that ANP receptor stimulation in brain microvessels is mediated, at least in part, by cyclic GMP. Receptors for the other vasoactive substances that were tested, if they exist in rat cerebral microvessels, are not related to cyclic GMP.

409.11
ATRIAL NATRIURETIC PEPTIDE STIMULATES GUANYLATE CYCLASE ACTIVITY IN ISOLATED RAT BRAIN MICROVESSELS. P. Hamvour*, and S.I. Harik (SPON: R.B. Daroff) Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

We tested the effect of several putative vasoactive substances, mostly peptides for which there is evidence that their receptors exist in brain microvessels, for their effect on cyclic GMP generation in isolated rat brain microvessels. Bulk tests of isolated rat cerebral microvessels were incubated with atrial natriuretic peptide (ANP), 10^-10 to 10^-7 M; angiotensin II, 10^-10 to 10^-7 M; bradykinin, 10^-10 M; carbachol, 10^-10 M; and thrombin, 10^-10 M for a period of 10 min after which the reaction was stopped and the microvessels were extracted with perchloric acid and the content of cyclic GMP in the extract was assayed by radioimmunossay. In all experiments, the ability of sodium nitroprusside (10^-4 M) to increase guanylate cyclase activity was ascertained. Of all the agents studied, only ANP was potent in stimulating guanylate cyclase. This stimulation was dose dependent, with maximum stimulation at 10^-6 M. Our results indicate that ANP receptor stimulation in brain microvessels is mediated, at least in part, by cyclic GMP. Receptors for the other vasoactive substances that were tested, if they exist in rat cerebral microvessels, are not related to cyclic GMP.

We previously reported that when the optic nerve is crushed in an adult mouse and the retina explanted onto laminin a week later, ganglion extending neurites by 24h and were quite numerous by 1wk.

In this study, adult and embryonic retinal explants were made on astrocytes from primary cell cultures from 2- to 10-d-old mouse and maintained in culture 8d to 7wk. In some preparations oligodendrocytes were removed by shaking or by anti-GaC plus complement. Neurites were visualized by anti-neuromuscular immunohistochemistry. At 1wk, adult retinal explants had fewer and shorter neurites on these cellular substrates compared to laminin. In contrast, embryonic retinal explants outgrew numerous long neurites on these same cellular substrate as early as 2d even when numerous oligodendrocytes were present.

We suggest that mature ganglion cells may be deficient in their ability to respond to astroglia, that old astrocytes can support embryonic neurite growth and that oligodendrocytes may not be a major factor in regulating this response. (Supported by NS26750)

410.2 DIFFERENT ARBORIZATION PATTERNS OF NASAL AND TEMPORAL OPTIC FIBERS REVEALED BY IN VIVO CONFOCAL MICROSCOPY. A.L. O'Leary, E.S. Fraser, Dept. of Physiology & Biophysics, Univ. of Cal., Irvine, Irvine, CA 92717.

In lower vertebrates, the axons of the retinal ganglion cells form a topographic projection in the optic tectum. During the initial formation of the projection in Xenopus, the optic fibers from the nasal and temporal retina first overlap in the tectum and later sort out into a topographic map. Little is known about the growth and arborization of single fibers during this dynamic process. To follow these events, nasal and temporal retinal ganglion cells were labeled with the fluorescent carbocyanine dye "DiI" and their growth and arborization were directly visualized inside the living tadpoles. Three-dimensional images of identified terminal arbors were obtained daily or up to five days using a laser-scanning confocal microscope. The fibers grew into the tectal neuropil as relatively simple axons with large growth cones and then ramified to form extensive arbors through extension and retraction of branches. While fibers from the nasal and temporal retina both showed continual growth and remodeling, they showed dramatically different rates of caudal extension. The nasal fibers extended rapidly into new regions of the caudal tectum whereas the temporal fibers showed a much slower extension rate, remaining in the rostral tectum. In addition, while both nasal and temporal fibers extend branches as they first enter the tectum, the temporal fibers extend more branches at early stages. The nasal fibers don't increase their branch number until they have reached the caudal tectum. These different rates of extension may be due to differences in the anterior-posterior arborization of the cell membrane for the appearance of topographic order along the rostromedial axis of the tectum. (NSF BNS8608356)

410.3 MONOCLONAL ANTIBODY A82 TRIGGERS EVACUATION OF GROWTH CONE CONTENTS AND DISTO-PROXIMAL BULK REARRANGEMENTS OF AXONAL AXOPLASM IN GROWING AXONS. S. Eirenafin-Staun, E. Knoepf and V. Lemmon. Dept. of Physiology, SUNY at Buffalo, Buffalo, N.Y., and Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Monoclonal antibody A82 has been shown to recognize a class of O-acetylated ganglioside and to inhibit neurite outgrowth of chick in vitro (Soc. Neurosci. Abstr. Vol. 14. p. 272). Phase-contrast video-microscopic studies reveal that A82 (1:1000) induces a global evacuation of the contents of growth cones in regenerating retinal ganglion cell axons of goldfish explants in vitro. The onset of effects is rapid and signaled by an immediate cessation of elongation, a loss of lamellipodia and an emergence of a prominent filopodial morphology. A retrieval of axoplasm begins in distal filopodia, and the material accumulates at the base of the growth cone. With time, there is an en masse redistribution of accumulated axoplasm in the retrograde direction, such that distal evacuated segments measure 30-80 μm after an hour. Membrane filopodial ghosts mark the original site of attachment. D11 monoclonal antibody, which recognizes O-acetylated GD1 ganglioside (courtesy of Dr. Joel Levine), does not induce a global disto-proximal evacuation. Fluorescence microscopy shows very strong A82 and D11 immunofluorescence distributed uniformly over the surface of axons. After treatment of living axons with A82, followed by Triton X-100 extraction, immunofluorescence is punctate, indicating an apparent stabilization of some antigen. Chloroform-methanol extraction of fixed axons appears to remove major factor in regulating this response. (Supported by NS26750)

410.4 A MONOCLONAL ANTIBODY WHICH RECOGNIZES GROWING AXONS IN THE GOLDFISH VISUAL SYSTEM. Vielmetter and S. E. Fraser. Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft 7400 Tübingen FRG.

During the continuous growth of the goldfish retinotectal system, few ganglion cell axons are added at the peripheral retinal margin. The axons of these cells travel as a coherent, age related bundle through the optic nerve and tract (Stuermer et al. '81), and around the dorsal and ventral margin of the tectum (Stuermer and Easter '84). These young axons are specifically recognized by a new monoclonal antibody, 587.

This antibody was obtained by immunizing mice with glycoproteins extracted from cell surface membranes of adult goldfish retinal axons, and purified with lentil lectin and WGA affinity chromatography. On frozen sections, 587 stains axon bundles in locations identical to the position of the additional cell mass. After optic nerve section, the retinal axons regenerate. Most or all regenerating axons are 587 positive distal to the cut, in the tract, and in the retinorecipient layers, SO and SFGS, of the tectum. Unfixed axons in the tract and growing on laminin, are stained by 587 throughout their length including their growth cones and filopodia. Staining with 587 does not require fixation or permeabilization of the cell membrane, indicating that the antigen is exposed on the cell surface. On western blots, 587 recognizes a protein of an apparent molecular weight of 200 kD. The antigen therefore is different from the antigen recognized by κ-N-CAM (Baselmeyer et al. '89).

Thus the 587 antigen is a cell surface protein which appears to be specific for growing retinal axons in fish.


Goldfish retinal axons regenerate after optic nerve transection (ONS). Mice were immunized with proteins which were extracted from cell surface membranes of the regenerating goldfish optic nerve and reconstituted in liposomes. We obtained an antibody, M802, that recognizes regenerating retinal axons in the retinotectal pathway.

On frozen sections of normal fish, M802 staining is restricted to cell surface membranes of the regenerating goldfish retina and reconstituted in liposomes. We obtained an antibody, M802, that recognizes regenerating retinal axons in the retinotectal pathway.

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Retinal axons and their growth cones growing from retinal explants in vitro carry M802 positive staining throughout their entire length. M802 staining does not require fixation or permeabilization suggesting that the antibody binds to the epitope on the axonal membrane. On immunoblots M802 recognizes a band of an apparent molecular weight of 50KD.

Whether M802 interferes with axonal regeneration is tested in a special in vitro assay (Vielmetter and Stuermer, Neurosci. Abstr. 1988).

410.6 EARLY DEVELOPMENT OF THE POSTOPTIC COMMISSURE IN ZEBRAFISH EMBRYOS. S.W. Wilson and S.S. Easter Jr. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

The zebrafish hatches at about 2.5 days post-fertilisation. We are studying the embryonic development of axon pathways in the brain. At 24 hours post-fertilisation the major pathway in the postoptic commissure is the striatus-shaped postoptic commissure (PoC) and its associated tract (tPoC). We have made small injections of HRP into the PoC at 24-27 hours of development (survival times were 10 minutes or less) to retrogradely label those neurons contributing to it. They are: 1) axons of 2 or 3 dorsal diencephalic neurons along the tPoC, and 2) a small population of bilateral hindbrain neurons that ascend through the ventral midbrain. Anterograde labeling of axons with orange fluorescent dyes revealed three other contributors to the tPoC that do not deccussate in the PoC (at this stage). They are: 1) axons of 2 or 3 dorsal diencephalic neurons just deep to, or perhaps within the anlagen of the epiphysis, 2) descending telencephalic axons, and 3) axons of midbrain neurons that enter the tPoC from the posterior commissure.

The tPoC intersects with three other tracts. At these intersections, the morphologies and trajectories of axons entering the tPoC have changed, hipotically, we have interpreted these changes in the context of axonal pathfinding. Supported by NATO (BSB1201) and NIH (EY-00168).
410.7 DEVELOPMENT OF FIN NERVES IN WILD-TYPE AND MUTANT FISH EMBRYOS. H. Okamoto* and J.Y. Kwada. (SPO: B. Oakley). Dept. of Biology and Gerontology, U. of Michigan, Ann Arbor, MI 48109.

The pectoral fin of the Japanese Medaka fish is a simple limb consisting of two muscles. We have studied axonal outgrowth by fin motor neurons and tested the role of the fin bud for axonogenesis by these neurons by labelling them in wild-type and mutant embryos with an monoclonal antibody to acetylated tubulin (G. Piper, A. Fuller, J. Cell Biol. 101:2085, 1985) which labels all embryonic axons. Fin axons originate from S1-S4 and project ventrally between the axial muscles and the notochord to the ventral surface of the axial muscles where they turn and extend laterally in between the muscles and the pronephros. Here the axons turn: S1 axons posterior, S4 axons anterior, and S2 and S3 axons extend lateral so that they fasciculate with each other to form a plexus. From the plexus axons emerge to invade the nascent fin muscles. To see if the outgrowth of these axons are dependent on the presence of the fin bud, we analyzed axonal outgrowth both in embryos in which a fin bud was ablated early in development and in the "pectoral finless" mutant whose fin bud become arrested at the earliest stages of development. In both cases the fin axons fasciculated to form a plexus but failed to extend beyond the plexus. Therefore, plexus formation is independent of the presence of the fin bud, but establishment of the nerve requires a normal fin bud. Supported by grants from NIH, March of Dimes, and the Office of VP for Research at U. of Michigan.


We have begun to study pathfinding by growth cones in the brain of zebrafish embryos by methods which allow one to analyze the development of identified neurons. The embryonic zebrafish brain consists of a small set of axonal tracts which is established by a relatively small number of identifiable neurons found in discrete regions. These identified neurons have axons with stereotyped trajectories which are located in a cell specific subset of the existing pathways. We have examined pathfinding by the growth cones of one of these identified neurons and found that these growth cones extend along a cell specific route from the beginning of axonogenesis. Growth cones of neurons in the nucleus of the posterior commissure, a dorsolateral nucleus near the border of the diencephalon and mesencephalon, extend ventrally to the anterior tegumentum. At this site several different pathways meet, yet these growth cones always extend posterior into one of the pathways. This may indicate that identified growth cones select their pathways at such intersections in order to reach their targets in the brain. The early stereotyped trajectories of other identified neurons open up the possibility that this may be a general feature of pathfinding in the zebrafish brain. Supported by grants from NIH, March of Dimes, and Office of VP for Research at U. of Michigan.


We have followed the axonogenesis of 4 of the 5 earliest neuronal types found in the zebrafish embryo by labelling cells with a variety of intracellular dyes, application of axonal tracer dyes, a monoclonal antibody against microtubules which labels all embryonic axons, and a monoclonal antibody which only labels 2 of the 5 groups of axons extending their growth cones. These neurons (RB, DLA, CoPA, and VLD) all project growth cones from the ventral halves of their somata. Subsequently these growth cones select cell-specific pathways to reach their targets in the CNS. Pathfinding by CoPA and VLD is interesting since initially both growth cones extend towards the floor plate, a single row of cells at the ventral midline. The CoPA growth cone crosses the ventral midline by extending under the floor plate and turns anterior, while the VLD growth cone comes into the immediate vicinity of the floor plate but never crosses the midline and turns posterior. These findings are interesting since in the rat embryonic cord the floor plate can specifically attract commissural growth cones (Tessler-Lavigue, et. al., Nature 336:775, 1988). We are presently investigating whether the floor plate is necessary for the turns made by these growth cones and whether it prevents the VLD growth cone from crossing the midline as well as attract the CoPA growth cone. Supported by grants from NIH, March of Dimes, and OVPR at UM.


As a basis for an analysis of axonal pathfinding we have characterized the cellular anatomy of the simple spinal cord of developing zebrafish with intracellular dye injections and axonal tracers. The five groups of embryonic axons have been distinguished based on somatic position, axonal trajectory, and time of axonogenesis (Co-commisural, Ci=centro-frontal, L=longitudinal, A=ascending, D=descending). There are 3 classes of Co-cells; early primary (CoPA), later secondary (CoSA), and bifurcating (CoB). Ci-cells are similar to Co-cells but have ipsilateral axons and fall into 2 classes (CIA and CID). The L-cells consist of dorsal (DLF) and ventral (VLF) cells. Also there are Rohon-Beard (RB) and motor neurons. These cells account for 50-75% of the 80-80 neurons per embryonic spinal segment. RBs and VLF establish an early dorsal (DLF) and ventral (VLF) fiber tract, respectively. CoPAs establish the commissural pathway. Most of the embryonic cell types are also found in larval (35) March of Dimes and RBs project to supraspinal levels (di-/mesencephalon and hindbrain, respectively). EM shows that the DLF and VLF are established by a small number of axons at 16-20 h. At stage only scattered axonal profiles are found in the intermediate lateral margin (presumably Co-axons). At 25-40 h addition of new axons, both to presynaptic bundles and at intermediate levels, creates a continuous marginal zone. Tectospinal axons establish a distinct ventral medial bundle (MLF) and commissural tracts are evident at 48 h. Supported by grants from NIH, March of Dimes, and OVPR at UM.

410.11 MOTOR INNERVATION OF DORSOVENTRALY REVERSED WINGS IN CHICK/QUAIL CHIMAERIC EMBRYOS. M. Ferns* and M. Holladay. (SPO: E. Thomas). Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

During the development of motor innervation of the chick limb, lateral and medial musculature are innervated by different sets of motor neurons. Some of the axons have been shown to project selectively to muscles of dorsal and ventral origin respectively. We have examined the axonal guidance cues involved in this process. We have assayed the axonal growth of bud forming section in wild type (DVT) and in mutant embryos (DVT-). We have used a variety of intracellular dyes, application of axonal tracer dyes, a monoclonal antibody against microtubules which labels all embryonic axons, and a monoclonal antibody which only labels 2 of the 5 groups of axons extending their growth cones. These neurons (RB, DLA, CoPA, and VLD) all project growth cones from the ventral halves of their somata. Subsequently these growth cones select cell-specific pathways to reach their targets in the CNS. Pathfinding by CoPA and VLD is interesting since initially both growth cones extend towards the floor plate, a single row of cells at the ventral midline. The CoPA growth cone crosses the ventral midline by extending under the floor plate and turns anterior, while the VLD growth cone comes into the immediate vicinity of the floor plate but never crosses the midline and turns posterior. These findings are interesting since in the rat embryonic cord the floor plate can specifically attract commissural growth cones (Tessler-Lavigue, et. al., Nature 336:775, 1988). We are presently investigating whether the floor plate is necessary for the turns made by these growth cones and whether it prevents the VLD growth cone from crossing the midline as well as attract the CoPA growth cone. Supported by grants from NIH, March of Dimes, and OVPR at UM.

410.12 COLLATERAL BRANCHING DURING DEVELOPMENT OF LIMB INNERVATION IN THE CHICK WING. M. Holladay & M. Ferns*. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The initial pattern of innervation of the limb bud has been reported to be highly specific, with only few instances of segmentally erroneous projections being detected. One possible form of refinement of this pattern that has received little attention, is collateral branching of axons to divergent targets. Axons projecting in either the dorsal radial or ventral brachialis inferior nerve trunks in the wing were retrogradely labelled by direct HRP injections at St 26-28, shortly after the formation of muscle nerve branches, and detected immunohistochemically in paraffin sections with the sensitive peroxidase anti-peroxidase technique. Collaterals of retrogradely labelled axons were consistently observed emerging from the dorsal nerve trunk proximal to the injection site. Collaterals were present in moderate numbers as compared to the number of labelled axons and extended varying, but often considerable distances (200-500µm ) into the muscle nerves. Collateral branching at the plexus where the pathways to dorsal and ventral muscle masses diverge was never observed. Therefore, whereas axonal branching at the muscle nerve branches may reflect localized regions of increased (but specific) subunit adhesion, differing guidance mechanisms are perhaps operative in the dorsovenral pathway choice.

It remains to be determined whether such projection of collaterals to multiple targets involves motor and/or sensory axons, and what is the time course and mechanism of their presumed selective elimination. It appears however that axonal growth cone guidance and elimination mechanisms are not mutually exclusive, but functions, formation and elimination, require accuracy and resolution, producing some imprecision in the initial pattern of projections. (Supported by NIH NS 25340).

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410.13

HEX-1 LABELS DEVELOPING XENOPUS NEURONS AND GROWTH CONES. P.W. Nordlander, Dept. Oral Biol., Case West Reserve Univ. Sch. Dent., Case Western Reserve University, Cleveland, OH 44106.

The monoclonal antibody HEX-1 has been widely used to identify migrating neural crest cells. The present study examines the expression of the HEX-1 epitope in the developing nervous system of Xenopus embryos and larvae as viewed in wholemounts. In these preparations HEX-1 gives a readily accessible overview of the developing fiber scaffolding from which much of the anti-HRP marking of the earliest neural elements of Drosophila. The antibody was found to mark developing neurons from the time of initial axonal outgrowth. It appears on the surfaces of cell bodies, axons, and axonal growth cones of identifiable tracts. In transients and its distribution follows a rostrocaudal developmental gradient: In a pattern consistent with observations from earlier studies using neuronal tracers and electron microscopy.

The association of this epitope with the surfaces of developing neurons suggests a function in axonal outgrowth. The author thanks E.Jaszczak for technical assistance and S.Landis for HEX-1. This work was supported by NS 18873.

410.15

EXPERIMENTAL EVIDENCE THAT TARGET MATURITY CAN INFLUENCE AXON OUTGROWTH IN VIVO. K. W. Tosnev, Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Axial cutaneous nerves emerge from the sensory ganglia several days after their counterparts that innervate cutaneous targets in the axial cutaneous nerves is temporally correlated with the maturity of the epidermis. Cutaneous nerves will form before stage 26 if their normal target is replaced with older or younger epidermis from quail and determining whether subsequent outgrowth of axial cutaneous fibers is proportionally advanced or delayed in time. Preliminary results indicate that the formation of axial cutaneous fibers is temporally correlated with the maturity of the epidermis. Cutaneous nerves will form before stage 26 if their normal target is replaced with older epidermis. This stimulation of outgrowth is not likely to be an artificial result, since younger target can suppress outgrowth. In addition, a small lateral patch of more mature epidermis can elicit formation of a novel cutaneous branch from the distal spinal nerve. The mature epidermis stimulates outgrowth despite the fact that it is usually well beyond filopodial reach. These results are consistent with a relatively long-distance cue emanating from the epidermis that attracts sensory growth cones. Alternatively, the epidermis may change or condition the more proximal pathway, rendering it more suitable for axonal regeneration. These preliminary results provide the first experimental evidence that temporal alterations in the target may contribute to the control of axonal outgrowth. Supported by NIH grants NS-21308.

410.17


The polysialic acid (PSA) moiety of NCAM can regulate the strength of cell-cell interactions mediated by either NCAM or by G4/L1. We previously found that the strength of axon-axon adhesion due primarily to G4/L1 has a strong influence on the degree and spatial pattern of nerve branching in embryonic muscle. Furthermore, in vivo inactivation of G4/L1 with specific antibodies produced changes in the nerve pattern similar to those following DTC-induced immunoneurotoxic activity. We report here that activity block does not decrease the actual level of G4/L1; however, it does cause a large increase in PSA levels in nerve, which we propose indirectly decreases G4/L1 function. This increase in nerve PSA can account for most of the inactivity-induced changes in branching, since PSA receptor, an endoneuraminidase prevents these changes in DTC-treated embryos. Since antibodies to G4/L1 but not to NCAM effectively reverses the changes produced by PSA, we conclude that PSA is the active ligand most responsible for the PSA-regulated changes in nerve branching. During normal development these changes, which are associated with a temporal-spacial pattern consistent with its postulated role in nerve branching. Supported by NIH grants NS 19640 and HD 18369.

410.14


Merkel cells may serve as targets for a specific class of cutaneous sensory nerve. The basis for this relationship is unknown. Trpionic factors promoting sensory neurites on this target have not been identified. NGF, a trophic factor promoting survival of neurons innervating this target, has been demonstrated in epithelium. The cellular source of this NGF remains unknown.

Primary cultures of dissociated epithelium were obtained from E20 to E24 buccal pads. Morphologically distinct cells were identified in vitro as Merkel cells based on their labeling with markers that specifically label Merkel cells in situ. These same cells were disassociated by their unique expression of NGF-like immunoreactivity in vivo, implicating Merkel cells as a source of NGF.

Many sensory neurons require NGF for survival, while sympathetic neurons universally depend upon NGF for survival. Serum free medium (SFM) supplemented with NGF promoted the long-term survival of sympathetic; but not sensory neurons. In contrast, cultures of epithelial cells containing Merkel cells promoted long-term survival of both sensory and sympathetic neurons. In these cocultures, antibodies against NGF reduced sensory neuron survival by 65% and reduced sympathetic neuron survival by 90%. Thus, NGF is necessary but not sufficient to promote long-term survival of sensory neurons in SFM.

Sensory nerves innervate the epidermis in situ, while sympathetic nerves do not. A similar discrimination was observed in vitro. Growth cones from 70% of sensory neurons were innervated associated with Merkel cells in cocultures examined 48 hrs after neurons were added. In contrast, only 10% of sympathetic nerves were in contact with Merkel cells. The fraction of sensory neurons contacting Merkel cells continued to increase with time in culture, while the fraction of sympathetic neurons contacting Merkel cells did not.

410.16

DEVELOPMENT AND INNERVATION OF THE PRIMARY ABDOMINAL MUSCLE IN EMBRYONIC XENOPUS LAEVIS. K.L. Lynch* and S.E. Fraser. (SPPY, R. Lewis) Dept. of Anat., Unis. of Calif., Davis, CA 95616-4799 and Dept. of Physiol. & Biophysics, University of California, Irvine, CA 92717.

The primary abdominal muscle of Xenopus develops during embryonic stages 31 to 40, from cell clusters that bud from the ventral lateral margins of trunk myotomes II to VIII. Because of its thin flat shape, regular segmentation and superficial position, the abdominal muscle is a useful subject for studies on neural migration, muscle morphogenesis and differentiation, and interactions between myogenic, extracellular matrix components, and the nerve fibers that innervate the muscle. The undifferentiated cells from which the abdominal muscle develops migrate ventrally from the myotomes as clusters of tightly adherent cells, a relatively unusual form of cell migration. We have used the fluorescent tracer Dil to label the clusters and record their movement by time-lapse videomicroscopy. The tracer also enabled us to identify the most rudimentary of the myotomal buds as the source of the muscle fibers in the geniohyoid muscle. Silver stains, electron microscopy and enzyme histochemistry revealed that the first motor fibers to the abdominal muscle. From the ventral rami of spinal nerves 2 through 9, initially follow the paths of the cell clusters from which the muscle develops. When the cells of the clusters differentiate and fuse to form myotubes, however, the motor axons are in a localized deep to the narrow zones between adjacent muscle segments. Fibers from spinal nerve 2 innervate the first segment of the abdominal muscle and the geniohyoid, consistent with the origin of the myogenic cells.

These data confirm and extend previous data on this system. With knowledge of the chronology and features of the abdominal muscle's development, we are in a position to manipulate it to explore underlying morphogenetic mechanisms.

410.18

TIME-LAPSE STUDIES OF ENCOUNTERS BETWEEN SENSORY NEURON GROWTH CONES AND NEURITES. M.G. Horig and S.M. Burden*. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

We are interested in elucidating the mechanisms by which sensory neuron growth cones acquire their target cells, which is the result of the interaction between the growth cone and the target cell. We are investigating this interaction both in vivo and in vitro using time-lapse video recording. Our work has shown that the growth cone interacts with target cells in a variety of ways, including crossing, branching, retracting and even merging with the target cell. These interactions are mediated by specific neurotrophic factors, such as nerve growth factor (NGF), which are specific to the target cell.

We are also interested in the role of the extracellular matrix in the guidance of sensory neuron growth cones. We have shown that the extracellular matrix can influence the growth cone's ability to navigate through the tissue. We are currently investigating the effects of different extracellular matrix components on the growth cone's behavior.

Finally, we are interested in the role of the guidance molecules in the development of sensory neurons. We have shown that the guidance molecules are specific to the target cell and can influence the growth cone's ability to navigate through the tissue. We are currently investigating the role of these molecules in the development of sensory neurons.

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AXON INITIATION BY DORSAL ROOT GANGLION NEURONS IN VITRO. C.L. Smith and E.M. Munro*. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD.

Axon formation signals the initiation of a neuron's differentiation and the beginning of a large increase in neuronal volume. We are investigating the intracellular changes underlying this process. Sensory neurons from chick embryos were dissociated and grown on polyethylene-coated glass coverslips in defined medium with nerve growth factor to promote neurite outgrowth. Live cultures are examined with phase-contrast microscopy. Neurons are collected from the dissociation and grown in an incubator. Immediately after plating, some neurons retain a remnant of their original axon but, within 1 to 2 hours, most neurons appear rounded and no axon is visible. These axonless neurons extend a flat, vein-like lamellipodium evenly around their circumference. Filopodia protrude from the lamellipodium. In low density cultures, neurons can retain this shape for at least 48 hours. However, neurons can be stimulated to form neurites by the addition of non-neuronal cells. Contact with the non-neuronal cell is typically made by a filopodium. The adjacent lamellipodium then elongates and thickens, and its tips grows along the non-neuronal cell. During this period, the lamellipodium between the developing axon and the soma remains thin, allowing imaging of intracellular organelles such as mitochondria, endoplasmic reticulum and microtubules. Epifluorescence imaging of organelles stained with fluorescent markers is also feasible. Thus, the separation of events in the organization of the movements of organelles and cytoskeletal elements during axon formation.


Involvement of excitatory transmission at NMDA receptor within the substantia nigra pars reticulata (SNR), entopducular nucleus (ENP) and ventrolateral nucleus of thalamus (VLTN) in the high pressure neurological syndrome (HPS) (tremor myoclonus convulsion) was studied in rats. Focal injection of NMDA (1-10 nmol) into each structure resulted in lowering the threshold pressure for the initiation of tremor and convulsions in hyperbaric conditions. Injection of APH injections into the SNR. The same injection into the EP was best in protection against tremor (48%). VLTN injections produced less pronounced effects but these were statistically highly significant.


We studied the stages of entry into status epilepticus in the lithium-pilocarpine seizure model with the 1-2-deoxy-glucose (2DG) method with the aim of determining the ana­

tomic sites of seizure origination and subsequent spread. Status was induced in 16 LIGIC-pretreated rats with pilo­

carpine, 20 mg/kg IP. 2DG, 40 μCi IV, was given either 1) at the beginning of discrete seizures (D), 2) during waxing-and-waning seizures (WM), 3) during continuous convulsive seizures with either fast- and slow waves on EEG (FS); or 4) fast continuous spiking (FC). 10 min later the brain was removed; cut sections were exposed to X-ray film. The resulting autoradiographs revealed that the most restricted patterns of seizure-induced hyperme­
tabolism occurred in D and WM subjects with minimal con­vulsive behavior. Intensive activation of limbic, rostral neocortical and thalamic areas. In FS, forebrain activation was global but maximal in orbitofrontal cortex and nearby orbitofactory areas. In FC, forebrain recruitment was completed. These results suggest that in this model, seizure activity originates in orbital cortex and nearby orbitofactory areas, then recruits successive anatomic shells.
NON-CONVULSIVE STATUS EPILEPTICUS PRODUCED BY ETHYL ETIOCOCCAYACINE AND PILOCARPINE CO-ADMINISTRATION. T. J. McCulloch. Intracellular Electrophysiology, Department of Pharmacology and Toxicology. University of Maryland School of Pharmacy, Baltimore, MD 21201.

Since convulsions in animals are thought to have a role in the regulation of seizure threshold and postsurgical activity, we evaluated the effects of the kappa receptor agonist ethyl etiochocamine (EEC) on pilocarpine-induced seizures in rats. Pilocarpine (100 - 250 mg/kg) produced seizures, but not convulsions. EEC (4 - 8 mg/kg; ip) caused EEC burstng and sedation but no convulsions. Administration of EEC (4 mg/kg) 5 minutes prior to pilocarpine (100 - 150 mg/kg) consistently resulted in the onset of status epilepticus (SE) in female and male rats within 20 - 25 min. EEC/pilocarpine induced SE was relatively mild compared to the high dose pilocarpine (600 mg/kg). SE was nonconvulsive throughout the 3 hour observation period; some rats showed clonic convulsions after about 2.5 hrs. Histological examination revealed mild pathology in the amygdala, piriform cortex, CA1 and CA3 of rats after three hours of SE. Preliminary Results suggest age-related differences in sensitivity to the SE produced by EEC/pilocarpine co-administration. These results provide further evidence for a role of opioid peptides in seizure mechanisms in general and in particular, status epilepticus. (Supported in part by NIMH R01 50670)

VENTRAL HIPPOCAMPAL DENTATE GRANULE CELL LESIONS POTENTIATE CONVULSIONS INDUCED BY A MU OPIOID RECEPTOR AGONIST. P.H.K. Lee and J.S. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

The present study investigated the role of hippocampal dentate granule cells on convulsions and wet dog shakes (WDS) induced by PL017, a mu opioid receptor agonist. PL017 (5 µg) was injected into the ventral hippocampus of rats 14 days after unilateral or bilateral colchicine (10 or 20 µg) lesions of ventral hippocampal dentate granule cells. PL017 injected into control (ACSF-treated) animals produced convulsions and numerous muscles twitches for less than 1 min. PL017-induced WDS were significantly reduced in unilateral COL-pilocarpine-treated rats, and completely inhibited in COL-pilocarpine-treated animals since status epilepticus was observed in both unilateral and bilateral COL-pilocarpine-treated rats but not in control animals. Furthermore, PL017 induced widespread, seizure-related damges of CA3/CA1 pyramidal cells in COL-pilocarpine-treated rats, but not in control animals. These results suggest that dentate granule cells in the ventral hippocampus are essential for the elaboration of WDS. However, these neurons may play an inhibitory role in the spread of seizure activity within the hippocampus or limbic structures.


Intracellular recordings were obtained from the entorhinal cortex (EC) and extracellular recordings were obtained simultaneously from the hippocampus (HIP) in perfused (O2) and in vivo (pineal body) guinea-pig brain. Extracellular stimulation of the EC evoked a transient potential in EC neurons (and sometimes an antidromic potential) and evoked a postsynaptic EPSP-EPSP, in the HIP which facilitated at 1 Hz. Higher frequency stimulation evoked ictal-type seizure discharges. Intracellular recordings in EC neurons indicated that epileptic discharges were composed of a train of paroxysmal depolarizing shifts (PDS) superimposed upon a slow depolarization. EC PDS's were synchronously distributed in population bursts in the HIP, as ictal discharges slowed and then blocked, PDS's occurred independently in the EC and HIP and extracellular stimulation increased synchronous activity in both the HIP and EC. Therefore reciprocal excitatory connections between the HIP and EC could be important in synchronizing ictal discharges.


Destruction of dentate granule cells (DGC) abolishes wet dog shakes (WDS) elicited by kainic acid (Grimes et al., J. Neurosci. 8:256, 1988) and induced by kindling of the entorhinal cortex (Bush and McNamara, Exp. Neurol. 12:102, 1986). However, the effect of destruction of DGC on seizure activity is not clear (cf. Grimes et al., ibid vs Okazaki and Nadler, Neurosci. 26:763, 1988). Previous studies suggested that DGC lesions did not affect the threshold of the dorsal (D) and ventral (V) hippocampus. We compared the effects of destruction of D vs V-DGC on WDS and seizures (S). D destruction completely abolished WDS (x = 1 ± 3.5) compared to control animals (x = 9 ± 3.5). However, both V and IM-DGC lesions lowered S thresholds. S threshold was defined as the current required to elicit rearing accompanied by fore-limb extension. The effect on S threshold was especially pronounced 8 weeks post colchicine lesions in the hippocampus where 7 of 10 animals exhibited seizures at 1 mA or less. Examination at even longer time intervals is thus warranted.

THURSDAY AM

EPILEPSY: BASIC MECHANISMS III

EFFECTS OF NORADRENERGIC AGONISTS MICROINJOINED INTO THE PONTINE RETICULAR FORMATION ON MAXIMAL ELECTROSHOCK-INDUCED SEIZURES IN THE RAT. M.L. Lanker and R.A. Browning. Dept. Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

Recent research has implicated the mesencephalic and pontine reticular formation in the propagation of generalized tonic seizures. Specifically, inhibition of the tonic components of generalized seizures [e.g. maximal electroshock (MES), pentylenetetrazol & audiogenic] has been observed following lesions of the mesencephalic pons or midline pontine (ROI) which include the superior cerebellar peduncle (Sempere et al., Brain Res. 469, 1988). Furthermore, considerable evidence indicates that norepinephrine (NE) inhibits the tonic components of MES-induced seizures. Therefore, we investigated the effects of noradrenergic agonists (NE, phenylephrine (PE) and isoproterenol (ISO)) into the ROI on tonic hindlimb extension (T) in the MES test.

Sprague-Dawley rats were pretreated to ensure the presence of HLE in response to MES. The rats were bilaterally implanted with 23 gauge guide cannulae directed at the ROI. Two weeks after implantation the rats were infused with 0.5 µl NE (0.5, 1.0, 2.0 µg), PE (8.0, 16.0 µg), ISO (2.0 µg), or saline bilaterally and twenty minutes later subjected to transcorneal MES (0.2sec, 150mA). NE, PE and ISO in the doses used in the present study failed to alter the incidence, latency or duration of HLE compared to vehicle-injected controls.

These data suggest that neither alpha nor beta adrenergic agonists are effective in attenuating MES-induced seizures when directly applied to the ROI. Additional doses of ISO as well as an alphagonist are currently being examined.

THE CENTRAL MEDIAL INTRALAMINAR NUCLEUS: THALAMIC SITE OF SEIZURE REGULATION. J.W. Miller. C. Hall* and J.A. Ferrendelli, Depts. of Neurology and Pharmacology, Washington Univ. Sch. of Medicine, St. Louis, MO 63110.

This study demonstrates that the central medial nucleus (CeM) of the thalamus controls the threshold and expression of generalized seizures. Injection of the selective GABA_B agonist pipеридине-4-сульфоновая кислота (PDS) into CeM in neonatal or near the CeM significantly facilitated bicuculline-induced myoclonic and clonic seizures but had no effect on tonic seizures. In contrast, the selective GABA_A agonist (-)baclofen markedly facilitated all these seizure types and also altered the probability of tonic hindlimb extension (TBE) with the tonic seizure. Low doses of (-)baclofen (0.3 to 3 nanomoles) increased TBE occurrence from two to three fold, while higher doses did not. Both types of GABA agonists also depressed the animals' level of arousal. Systematic mapping of the midline thalamus with very small (-)baclofen injections (2 nanomoles in 0.05µl) revealed that injections centered in the CeM produced the greatest depression of arousal, and significantly lowered all seizure thresholds while injections centered in adjacent nuclei did not.

This work indicates that different GABAergic neural mechanisms in the CeM are responsible for modulating both the threshold and expression of various seizure types. The results of this study are best explained by the concept that the CeM is not a site of seizure origination or spread by rather acts via widespread connections to other brain structures involved in seizures. Supported by NIH grants NS01296 and NS 14834.
EPILEPSY: BASIC MECHANISMS III  
THURSDAY AM

411.11  
LONG-TERM ELECTROPHYSIOLOGICAL AND BIOCHEMICAL MONITORING IN THE RAT INFRA-AMYGDALOID TETANUS TOXIN CHRONIC MODEL OF EPILEPSY.  
An infusion of picogram amounts of tetanus toxin into rat amygdala results in spontaneous seizures peaking in severity and frequency at 5-10 days. We used awake, behaving male Fisher rats with chronic electrode implants for EEG, and guide cannulae for toxin infusions and in vivo microdialysis.  
Using in vitro and in vivo recording, we demonstrated increased multilaminar activity in epileptiform EEG activity, with spikes and seizures arising independently from the amygdala, hippocampus, and cortex; by the peak of the response the infused amygdala became the driving seizure focus, with secondary ictal observed consistently from hippocampus.  
Focal microdialysis demonstrated decreased basal and veratrine stimulated GABA levels, most consistent at the seizure peak, but appearing to persist consistently from hippocampus.

411.12  
INTRACEREBRAL MPTP PRODUCES EPILEPTIFORM ACTIVITY INDEPENDENT OF CATACOLINE RELEASE.  
Systemic administration of MPTP produces acute convulsions in all awake species studied. Mice with implanted hippocampal electrodes show EEG spikes and seizures after i.p. MPTP; this activity is dose dependent, attenuated by MAO, and exacerbated by diethyldithiocarbamate, suggesting dependence on intracerebral MPTP.  
Rats with implanted electrodes and guide cannulae were infused with MPTP or MPP+ into brain. Both compounds were epileptogenic when infused into amygdala or hippocampus. MPTP appeared factor-acting and more potent. Both amygdala and hippocampal infusion of MPP+ resulted in extracerebral discharges at lower doses (150 pmol to 60 nmol); doses of 3-6 umol MPP+ in hippocampus produced focal and behavioral seizures. MPTP-MPP+ responses were unaffected by reserpine (5 mg/kg for 3 previous days). No such abnormalities were observed after infusions (30 nmol) into substantia nigra, striatum, or deep perirrinal cortex.  
These results indicate a unique excitatory action of MPP+ in brain independent of its acute catacholine releasing effect.

411.13  
EMBRYONIC BEHAVIOR AS A MODEL FOR POSTNATAL EPILEPTIFORM ACTIVITY.  
Chick embryonic behavior is produced by spontaneous bursts of spinal cord neurons first described by Provine and associates (Brain Res. 29 (1971) p. 151; 41 (1972) p. 375; 45 (1972) p. 127.) Neurobiol. 8 (1977) p. 217). The bursts are synchronized with movement in motile embryos and motor nerve discharges in cutaneous preparations. The bursts usually begin with a high amplitude "initiating" discharge that is usually followed by a lower, longer amplitude, and more variable "afterdischarge." Before 7 days, bursts consist only of "initiating" discharges. Dual electrode studies indicate that bursts appear almost simultaneously along the rostrocaudal axis of the cord; bursts are initiated at varying cord loci and are then propagated throughout the remainder of the cord. The burstingness and transregional coupling of discharges are not typical of the postnatal cord.  
The transregionaly synchronized bursts of cord and perhaps other parts of the healthy embryonic CNS may provide a useful model of adult epileptiform activity that does not require the poisons, trauma, or kindling necessary in other model systems. Indeed, some epileptiform disorders may be a postnatal remnant or recapitulation of an embryo-like state caused by developmental disorder, trauma or disease.

411.14  
Y-HYDROXYBYTURATE (YGB) MODEL OF ABSENCE: CORRELATION OF Y-BUTYROLACTONE (YBL) AND GHB LEVELS IN BRAIN WITH EEG CHANGES.  
ICP and EEG electrode studies in adults and children indicate there is a close correlation between levels of GHB and YGB in brain and the frequency of absence seizures. We have confirmed these observations in the absence seizure prone rat.  
We postulate a mechanism in which YGB enters the brain directly from the gastrointestinal tract and then, by activating GABA receptors, decreases cortical neuronal activity, increasing the frequency and duration of absence seizures.

411.15  
INTRA-CELLULAR RECORDING OF THE SPONTANEOUS ACTIVITY IN DORSAL HORN INTERNEURONS INDUCED BY ELEVATED Ca2+ IN MOUSE SPINAL CORDS IN VITRO.  
In the present study, hemisected spinal cords with attached roots were prepared from 9-14 day-old mice and maintained in an interface chamber at 34.5 C. Suction electrodes were used to stimulate and to record from DR and VR. DM neurones were sought by intracellular microelectrode filled with 4M K-ascite. When [Ca2+] was 2 to 3.6 mM, the membrane potential was about 2 mV more negative than in control solution (1.2 mM) measured in the same 9 cells. The threshold of spike generation did not change. EPSPS evoked by DR stimulation become more prolonged in high [Ca2+]. In 2.4 or 3.6 mM [Ca2+], 17 out of 25 cells were spontaneously active, compared to 7 out of 17 cells in 1.2 mM [Ca2+]. Spontaneous activity in 6 cells was synchronous with the dorsal root discharges in elevated [Ca2+]. We conclude that only a part of the DH cell population is recruited into synchronized spontaneous activity in elevated [Ca2+]. Waves of depolarization of primary afferent terminals appear to be responsible for the synchronization. (Supported by grants NS17771, NS02633)

411.16  
HYPERAMMONEMIA PRODUCES SEIZURES BY NEURONAL DEPOLARIZATION.  
Hyperammonemia can cause seizures. These seizures are thought to be of spinal origin, and to be initiated by the inactivation of Cl-extrusion from neurons by hyperammonemia.  
To investigate the mechanism of hyperammonemia-induced seizures, we recorded extracellular potentials from spinal motoneurons in the lumbar enlargement in cats using the intracellular recording technique. We found that hyperammonemia decreased the extracellular Cl-concentration in the spinal cord, and that this decrease was associated with an increase in spinal motoneuron activity.  
These observations indicate that hyperammonemia depolarizes motoneurons and initiates spontaneous paroxysmal discharges. Since MPA, which inhibits Cl-extrusion, was ineffective in preventing seizures, it is likely that neuronal depolarization and not a decrease of post-synaptic inhibition, initiates the seizures due to hyperammonemia.
GABA SENSITIVE NIGRAL EFFERENTS MAPPED WITH 14C DEOXYGLUCOSE AUTORADIOGRAPHY IN RAT PUPS. E.F. Sperber, L.L. Brown D.M. Smith* & S.L. Moshé. Depts of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

The substantia nigra plays a role in the modification of seizures. In rats, nigral injection of bicuculline, a GABA Agonist, has an anticonvulsant effect. To identify the brain regions involved in the bicuculline-induced, nigral-mediated seizure suppression, we used 14C deoxyglucose autoradiography. A unilateral cannula was implanted in the substantia nigra of each of 9 rat pups (age 14 days). Two days later, they were infused with 100 mg/kg bicuculline. Thiry minutes later, the pups were injected with 10 µCi of DG, ip. Analysis of the autoradiograms demonstrated ipsilateral decreases in glucose utilization in the postrolandic frontal cortex, auditory, olfactory, posterior thalamic nuclei, dorsal striatum and frontal cortex of the bicuculline-treated pups as compared with controls. These results suggest that the bicuculline-mediated suppression of seizures in pups involves the retrolaminar and thalamic areas which can have widespread correlative influence.

CURRENT SOURCE DENSITY ANALYSIS OF PROPAGATING EPILEPTIFORM ACTIVITY IN RAT PERIRHINAL CORTEX REVEALS INVOLVEMENT OF INTRINSIC ASSOCIATION FIBERS. K.K. Kuchem* and L.B. Haberly, Dept. of Anatomy, Univ. of Wis., Madison, WI 53706.

The importance of perirhinal cortex (PC) in experimental models of epilepsy has been demonstrated by the discovery that injection of picomole amounts of convulsants in deep ant. PC evokes generalized seizures (Piredda & Gale, Nature 411.18). The correspondence in latency of peak dendritic currents during epileptiform activity to the period of synaptic events during this oscillation suggests that the latencies of the synchronized discharges of the afterdischarge are different from one seizure-like event to the next. Thus conventional signal averaging of these events is impossible. In order to begin a detailed analysis of these discharges we have developed an adaptive variable latency averaging procedure. The computer program detected a relative strong stimulus in layer Ib where association fibers from the ant. PC terminate throughout the PC and entorhinal cortex. A collapse was immediately achieved by synchronizing pyramidal cell firing and a subsequent high amplitude sink in layer Ib. An intriguing finding was that the latency between successive current peaks of epileptiform events was 20ms or integral multiples of 20ms at both ant. and post. sites.

CSD analysis of oscillatory responses to low strength shocks, resembling those recorded in response to odors, has revealed a stereotyped sequence of events that recurs during each cycle of a 20ms oscillation (Kuchem & Haberly, Soc. Neu. Abs. 14.118). The correspondence in latency of peak dendritic currents during epileptiform activity to the period of synaptic events during this oscillation suggests a possible link between the evolution of epileptiform activity in the PC and the dynamics of the normal response to odor stimulation. Supported by NICHD grant NS19865 to LBH and NSKA award NS08328 to KKL.


A sixteen channel multi-electrode (Otto Sensors, 150 µm contact distance) was used in the halothane anesthetized rat to record electrical activity from the dorsal hippocampus: area CA1 and Fascia Dentata. A current source density (CSD) analysis, for which the necessary conductivity measurements were performed, allowed the localization of current sinks and sources during tetanic stimulation at the Schaffer collateralis (SC): 10-50 Hz for 2-10 seconds. DC-measurements revealed a dendritic sink that increased in amplitude during the tetanus. This profile was compared with potentials evoked by single or double stimuli to SC or to Alveus fibers in Stratum Oris. From the same experiment we observed the electrical seizure that often followed the tetanic stimulation. Bursts during a seizure in area CA1 showed a dendritic sink in Stratum Radiatum and a somatic source in Stratum Pyramidal. Under these conditions conductivity corrections were made of marginal importance. In some animals a relative strong stimulus could lead to a spreading depression (SD) ending the seizure activity. In this case a strong dendritic sink is accompanied by a somatic source in CA1 pyramidal cells and in granular cells in both blades of the dentate. It be 7 seconds apart.

REDUCED ACETYLCHOLINE RECEPTOR EXPRESSION IN MUSCLES FROM CHRONIC ETHANOL-FED RATS. J.R. Held, S.T. Sayer* and J.A. McLane. VA Hospital, Hines, IL 60141 and Dept. of Biochemistry, Loyola Univ. Sch. Med., Maywood, IL 60153.

Our objective was to determine whether the gene expression of acetylcholine receptor (ACHR) protein is altered in muscles from an animal model of chronic alcoholism. Test rats received a nutritionally complete liquid diet containing 6.7% ethanol. Age and weight-matched control rats were pair-fed an isocaloric diet. After a 16 week diet period, soleus muscles were obtained, and total RNA and poly(A)+ RNA were isolated. Genes for Sarcoplasmic RNA binding and hybridized with a 32P-labeled complementary riboprobe to detect ACHR α-subunit mRNA levels. This riboprobe was prepared by RNA:DNA hybridization of a cDNA clone (RMA 407-12 obtained from J. Boulter) that encodes the ACHR α-subunit. Autoradiograms of these hybridizations revealed a variable degree of atrophy from slight to severe and occasionally necrosis was seen. Capillary alterations were similar. The differences between these groups could be explained because the biopsies were obtained from muscles with different dynamic properties and fiber composition. This work is supported by grants from NCI (CA 64037) and DCCD of UAE (E-03-17-86), Fundacion Polar and British Council.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

NEUROMUSCULAR DISEASE

ULTRASTRUCTURAL PATHOLOGY OF SKELETAL MUSCLE IN PARANOID-PLASTIC SYNDROME. A. Marquez*, Y. Blanc*, P. Tone*, H.J. Finol, J. Prieto* and L.R. Sosa*, Medicine and Sciences Faculty, Universidad Central de Venezuela and "Jose Ignacio Baldi" Hospital, Apartado 50587, Sabana Grande, Caracas 1050, Venezuela.

Patients suffering from cancer without muscle metastasis may present muscular weakness and wasting. In order to study the ultrastructural basis of these phenomena we examined muscle in cancer patients with gastric (n=4) and colonic (n=4) cancers and from quadrieps femoris of patients with bronchogenic car cancer (n=2). The types of degenerative lesions observed, biopsies from patients with gastric and colonic cancers presented slightly atrophied fibers mixed with many fibers with segmental necrosis, capillary alterations which included necrosis, and a mononuclear cell infiltration. The biopsies from patients with bronchogenic cancer exhibited a varied degree of atrophy from slight to severe and occasionally necrosis was seen. Capillary alterations were similar. The differences between these groups could be explained because the biopsies were obtained from muscles with different dynamic properties and fiber composition. This work is supported by grants from CDCH of UCV (E-03-17-86), Fundacion Polar and British Council.

Ganglioside (GD) produces chemical sympathectomy in rats (GUSB). We examined GUSB as a model of human sympathetic neuropathy. Rats (150-350 g) received 50 mg/kg of saline for 6-12 w. Subsequently, they were anesthetized (Inactin) and cannulated for mean arterial pressure (MAP) and heart rate (HR) recording. Tyramine (Ty, 30 nmol) and phenylephrine (Phen, 0.06 or 0.6 µM) were infused intravenously. Norepinephrine (NE) levels in plasma, femoral arterial blood (FA), superior caval venous (Scv), atrium, and sciatic and vagus nerves were measured by RIA.

As compared to controls, GUSB rats showed (a) lower basal MAP but normal Scv NE, (b) blunted responses to Ty in terms of increase in MAP (3.5±0.8 vs. 9.0±3.3 mm Hg; p<0.0001) and plasma NE (0.12±0.11 vs. 6.16±0.61 ng/ml; p<0.00002), (c) exaggerated pressor responses to Ph (e.g., with 0.06 µmol, 59.2±2.1 vs. 12.1±6.74 mmHg, p<0.001), and (d) lower NE levels (mg/kg) in all tissues (e.g., SCG: 0.5±0.16 vs. 1.7±3.17 pmol/kg, p<0.001). There was correlation between NE content in SCG and FA and responses to Ty and Phen, respectively. There was no correlation of NE levels in plasma and tissues.

Thus, (a) GUSB reproduces findings of human sympathetic neuropathy and (b) pharmacological responses, but not basal plasma NE, reflect the degree of NE depletion.

412.5 PATCH CLAMP MEASUREMENTS OF VISCOELASTIC PROPERTIES AND MECHANOELECTRICAL TRANSDUCTION IN DYSTROPHIC MUSCLE MEMBRANE. B.J. Cooper* and O.P. Hamill. Department of Pharmacology and Neurobiology, Cornell Univ., Ithaca, NY 14853.

Recently, the gene which is defective in Duchenne muscular dystrophy (DMD) has been identified and its 400 kD protein named dystrophin, has shown to be absent or abnormal in patients with DMD as well as in the canine model of the disease (CXMD). However, at this stage there is no direct evidence on the mechanism by which dystrophin actually maintains healthy muscle function nor why its absence results in a progressive muscular weakness. Based upon its subcellular localization in the sarcolemmal (PM) and plasma membrane (PM) fractions, we were interested in whether dystrophin plays a role in the stabilization of healthy muscle cells, if the absence, the membrane may become more fragile and leaky to ions such as Ca++. Another possible pathogenic feature is that there is abnormal activation of the strong activated (SA) Ca++ permeable channel that has been reported in cultured muscle cells. To test these ideas we carried out patch clamp measurements of acutely isolated adult muscle taken from the tibialis anterior of healthy and dystrophic muscles. It may either reflect a real absence in fully developed muscle, or a particularly low channel concentration. We have recently identified that dystrophin is required for the maintenance of a high affinity calcium channel in the sarcolemmal face of muscle.

412.7 ANTIBODIES FROM PATIENTS WITH LAMBERT-EATON SYNDROME RECOGNIZE BOTH PLASMA MEMBRANE AND CYTOSOLIC ANTIGENS IN CHROMAFFIN CELLS. M., P. Vielion*, K.K. Ward*, P.A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905.

We have previously reported the presence of neuromuscular junction (NMJ), synaptic, and peripheral nerve antibodies in patients with Lambert-Eaton myasthenic syndrome (LEMS). In this study we identified antibodies to both plasma membrane (PM) as well as cytosolic antigens in chromaffin cells (SC). We used Westerns blots of chromaffin cell fractions. Post-nuclear supernatant, post-microsomal supernatant (cytosol) and plasma membrane (PM) fractions, confirm the presence of a cytosolic antigen that is also present on the PM. One control IgG reacted with a protein also recognized by LEMS IgG (26,000 daltons) and was present on both PM and cytosol fractions. We were unable to detect SA channels in either healthy or dystrophic muscles, which may either reflect a real absence in fully developed muscle, or a particularly low channel density. Supported by the Cornell Biotechnology Program.

412.8 MOLECULAR CLOSING OF AN ANTIBODY THAT CROSS REACTS WITH DNA AND MYELIN FROM A PATIENT WITH CHRONIC LYMNHOCYTIC LEUKAEMIA AND PERIPHERAL NEUROPATHY. I. Tect*, D.C. De Vries, S. Studzinski, K. Waisman, M. Williams and F. Latt. Lab. of Neurology, Columbia-Presbyterian Medical Center, New York, NY 10032.

Evidence is emerging that primary systemic carnitine deficiency, a potentially lethal but curable inborn error of fatty acid oxidation, involves a cellular defect in carnitine uptake (Eriksson et al., Eur J Pediatr. 147: 662, 1988; Treem et al., Neuromuscular Disease Thursday AM.

Carnitine uptake was determined at carnitine concentrations between 0.1 to 50 µM. Non-specific uptake was determined at carnitine concentrations between 0.1 to 50 µM. Non-specific uptake of carnitine was determined at carnitine concentrations between 0.1 to 50 µM. Non-specific uptake was determined at concentrations of 10 mM. The two patients showed no significant uptake in the physiological range, implying a marked deficiency in the specific, high affinity, low concentration uptake mechanism. Both presumed heterozygous parents of patients #1 had normal carnitine substrate concentration Km values but significantly decreased Vmax and Vmax values for carnitine uptake (40% of controls). This, in addition to the reduced carnitine substrate levels in both parents of patient #1, supports an autosomal recessive inheritance pattern.

412.4 OXYGEN-FREE RADICAL (OFR) EFFECTS ON THE SCIATIC NERVE IN EXPERIMENTAL DIABETES. K.K. Ward*, N. Farzandi*, P.A. Low (SPON: J.P. Whisnant). Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905

We have previously reported the presence of neuronal, hypoxic, ischemic, impairment of the blood-nerve barrier and reduction of norepinephrine (NE) and 6-keto-prostaglandin F1α in chronic streptozotocin diabetic neuropathy (SN). We interpreted these findings as suggesting the involvement of OFR. We now report our studies on sciatic nerve conjugated dienes, hydroperoxides, NE, and malondialdehyde (MDA) in SN at 1, 4, and 12 months in male SD rats. Severe hyperglycemia was present throughout in SN. Conjugated dienes were consistently increased at all time points, hydroperoxides were consistently reduced, and MDA was not significantly different in SN when compared with controls.

These findings are consistent with the presence of OFR effect, and emphasizes the need to measure several indices.


The disease in the Mnd mouse is characterized by a progressive increase in motor dysfunction, leading to paralysis. Cytopathological changes in spinal motoneurons correlate with the symptomology. In particular there is a dramatic rearrangement of neurofilaments in the perikarya. These elements become marginalized leaving large areas in the cytoplasm absent of immunostaining with antibodies to phosphorylated, to non-phosphorylated or to core (phosphorylation independent) neurofilament epitopes, which appear to increase in volume with severity of the disease, are filled with inclusions that appear amorphous at the light microscopic level. Ultrastructurally, they assume a variety of forms; membrane bound vesicles filled with tubular profiles that are 25nm in diameter, sometimes containing paracrystalline arrays of undetermined substances; electron dense bodies with areas of fine lamellations; and ordinary lipofuscin. Light microscopic acid phosphatase histochemistry showed an increased, granular cytoplasmic staining in these neurons, commensurate with lysosomal localization. These data suggest that one aspect in the pathogenesis could include an increase in autophagocytic activity leading to an abnormal accumulation of intracellular inclusions. If the tubular profiles present in the inclusions represent microtubule degradation, this could compromise axonal transport and lead to cell degeneration.

(Supported by NIH NS52482 and the ALS Association)


Membrane-mediated excessive intracellular Ca accumulation (EICA) is a fundamental pathogenetic event associated with chronic muscle degeneration to Duchenne muscular dystrophy (DMD) (Bhattacharya & Johnson, Proc. 14th World Congress of Neurology, 88:1, 1989). EICA, necrosis, global muscle weakness, cardiomyopathy with EKG changes, and impaired mitochondrial function have been reported in the BIO 14-6 RH. Because of significant EICA in the CHF-146 strain DH (Soc. Neurosci. Abstr., 14:12, 1988), we studied the function of mitochondria (MIT) from cardiac and skeletal muscles of young (30-day) and old (>365-day) DH. CHF-148 strain normal hamsters served as controls. MIT were isolated and studied according to Thakar et al. (88A, 31:418-19, 1973). MIT from skeletal muscles of young DH were uncoupled (AOP/10M and RCR=1), whereas those from the older DH were relatively normal. Conversely, myocardial MIT from young DH were functionally normal, although those from old DH revealed significant impairment of oxidative phosphorylation. We conclude that muscle weakness and MIT dysfunction in DH is expressed at an early age in the skeletal muscle, whereas these changes in the ventricular myocardium are manifested later. This parallels the clinical course in DMD, and further justifies the use of CHF-146 strain DH as an animal model for the study of DMD. (Supported by NIH Grant AAR-38540 to SKB.)


The purpose of the study was to examine oscillations of the leg in normal and spastic subjects. Specifically, the oscillation was in the form of a clinical test called the "pendulum test" in which gravity was used to oscillate the leg of the subject. The motivation for studying the pendulum test was to determine if realistic aspects of spasticity and neuromuscular control could be incorporated into a description of the motion and to better understand the underlying mechanisms involved. Data of a specific trial of the test were used to fit the parameters of a linear second order system model by least square error algorithm in order to simulate the passive motion. The model of spastic motion had additional components accounting for the abnormal stretch reflex activation found in spastics. These modifications, corresponding in time to muscle activation included: increased stiffness (K) and damping (B) values; changes in the zero length of the lumped stiffness element; and timing of changes and gain of mechanical parameters related to the EMG recorded.

The results showed a linear second order model was an inadequate description of motion. First, the mechanical parameters (K and B) of the passive motion were found to be nonlinear. The nonlinearities included asymmetries for extension and flexion movements and amplitude dependence of K and B. These nonlinear properties were simulated in a model by making K and B a function of the amplitude of motion and the inverse of oscillation amplitude squared. Second, results from the spastic trials showed K and B did increase significantly, with a shift in spring zero length during stretch reflex activity. EMG recordings were useful in determining the timing and magnitude of these changes. A comparison of the model to other experimental data from the same subject showed the model could simulate different trials well; the variance accounted for by the model was usually over 90%. The analysis of the motion and model suggests that reflex thresholds for abnormal stretch reflex in spastics can be useful clinical measurements and obtained from the pendulum test.

Work supported by NIH grant NS-19331.

It is known that neurites from the disaggregated neural tube cells of Xenopus embryos show a striking orientation toward the cathode (negative pole) in response to weak DC electric fields applied to cells growing on tissue culture plastic (Falcon). Since the substrate is known to affect neurite initiation and may also play a role in neurite guidance in situ, we have extended these studies to include the field-induced responses of Xenopus neurites growing on various substrates. Cells were grown in electrical field chambers constructed from tissue culture plastic dishes that were untreated or coated with poly-L-lysine (PLL) or with an overlying layer of laminin (FL-L). Data were collected as photographs of each cell in the dish at the conclusion of the experiment. Neurites were analyzed with the aid of a digitizer interfaced with a microcomputer. Cells grown on PLL showed a strong orientation of neurites toward the anode, but neurites on PLL substrates grew toward the cathode. Initiation sites were predominately on the cathodal sides of the cell bodies, regardless of substrate. Control cells showed random neurite initiation and no overall growth on all substrates. Experiments are currently underway to investigate the effect of surface charge on field-induced neurite guidance.


Collagen is an important substrate allowing attachment of neurons and their neurites. We previously reported neurite extension on collagenase-etched collagen fibers. In this study, we investigate neurite extension on etched fibers using immunocytochemical techniques. Cortical cells from the brains of Sprague-Dawley rat fetuses at day 15 of gestation. Cortical cells were grown for 48 to 96 hours on coverslips containing etched collagen fibers. The etched collagen fibers were then fixed in a L-lysine, D-lysine, and L-lysine, D-lysine mix containing 1% formaldehyde followed by the addition of fluorescein-conjugated IgG. Immunofluorescence of neurites and neurite fascicles was detected along the long axes of etched fibers. We conclude that etched collagen fibers allow the attachment of neurites and direct their extension. Supported by a fellowship from the New Jersey Center for Advanced Biotechnology and Medicine, and a grant from NIH to R.M.G.

MORPHOLOGICAL FEATURES AND NEURAL DIFFERENTIATION OF NEUROBLASTOMA (N-2A) INDUCED BY REDUCED MEDIA SERUM CONCENTRATIONS. J. Rossi III, A.A. Messier*, R. Herder* and A. Callahan*. Naval Submarine Medical Research Lab., Biomedical Sciences Dept., Groton, CT 06340.

Previous studies demonstrated that cloned cells extended neurites when incubated in media with low serum concentrations. In these studies, these cells were cultivated in media containing 10% fetal bovine serum (FBS). After 24 hrs the cells were subcultivated in media containing 10%, 5%, or 1% FBS; or media containing 10%, 5%, or 1% JO-SERUM V (NSV). The cells were morphologically characterized after 24, 48, 72 and 96 hrs incubation. Reduced media serum concentration increased the proportion of differentiated cells. The neurite yields increased the length of the neurites, increased the number of varicosities on the neurites, and increased the number of varicosities on the neurites from the specific substrate. All measures NSV substitution reliably induced greater neuritogenesis than equal concentrations of FBS.
413.7  
GANGLIOSIDE ENHANCED NEURITE GROWTH: EVIDENCE FOR A SELECTIVE INDUCTION OF HIGH MOLECULAR WEIGHT MAP-2.

Neuroblastoma cells (clone S 26) maintained in serum free medium exhibit a progressive and significant induction of MAP-1a and Tau proteins, but not MAP-2; the time course of these inductions is highly correlated with an increase in microtubule mass which parallels neurite growth.

We examined the ability of Bovine brain gangliosides (BBG) to enhance the neurite outgrowth response of these cells. We report here that one effect of gangliosides is to selectively and dramatically induce the expression of MAP-2; our results also indicate a strong parallelism between this induction and the increase in microtubule mass which accompanies the growth of numerous, long, and highly branched neurites.

These observations suggest that MAP-2 induction in neuroblastoma cells may lead to a further differentiation of neurites equivalent to that observed in mature neurons.

Finally, our results indicate that gangliosides per se are not neuritogenic factors but rather substances capable of greatly enhancing cell derived influences which affect the neurite outgrowth response of neuroblastoma cells and the type of MAP that they express.

Supported by CONICET (PID 156) to A.G.

413.8  
TYROSINE KINASES DERIVED FROM NERVE GROWTH CONE GLYCOPROTEINS. K. Cheng*, R. Switala* N. Sahyoun.

Welcome Research Laboratories, Research Triangle Park, NC 27709.

Glycoproteins were isolated from neonatal rat brain growth cones by wheat germ agglutinin-affinity chromatography. Phosphorylation of poly glutamy ra revealed the presence of a tyrosine kinase activity (1 mmol/min/mg protein) which required the presence of both MgCl2 and MnCl2. Tyrosine phosphorylation of several endogenous polypeptides was also observed in the presence of MnCl2. Enzyme substrates included tubulin and a Mr 115,000 polypeptide whose electrophoretic mobility could be altered by the presence or absence of reducing agents. Insulin stimulated the phosphorylation of a Mr 90,000 polypeptide while IGF-1 elicited phosphorylation of 90,000 and 97,000 polypeptides corresponding to the #-subunit of the insulin and IGF-1 receptors, respectively. Tyrosine phosphorylation of endogenous substrates was confirmed by phosphomono acid analysis, alkaline hydrolysis and immunoprecipitation with phosphotyrosine antibodies. In contrast to growth cones, synaptosomal glycoproteins were relatively lacking in protein tyrosine kinases, suggesting that the expression of these enzymes may be regulated by the stage of neuronal development, and that they may play a significant role in growth cone transmembrane signalling.

413.9  
Mathematical Analysis of Growth Cone Motility
H. M. Buj/Image* and R. N. Pittman. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19101-6084.

Mathematical modeling is a potentially valuable, but as yet, little used tool for investigating both cellular and molecular mechanisms of neurite outgrowth. Since neurite outgrowth depends critically on growth cone motility, a logical starting point in the mathematical description of neurite outgrowth is the quantitative analysis of growth cone movement. We have initiated a mathematical modeling study of growth cone motility that applies two methods previously used successfully to describe motility in other cell types:

1) measurement of the root-mean square speed and persistence time of individual growth cone (see: G.A. Dunn, Agents Actions [Suppl] 12 14, 1983)
2) measurement of growth cone shape and orientation based on the moments of growth cone area (see: G.A. Dunn and A.F. Brown, J.Cell Sci. 82 313, 1986)

Data is being obtained from primary cultures of rat sympathetic neurons grown on laminin using both time-lapse videomicroscopy of live cultures and morphometric analysis of fixed cultures. These results form the basis for a stochastic model of growth cone motility describing the trajectories traced out by individual growth cones. Such a model can be used to explore the effects of different homogenous substrates, heterogeneous substrates and trophic factors on neurite outgrowth and guidance. (Supported by the Whitaker Foundation)

413.11  
DISTRIBUTION OF A 33KDA PROTEIN IN CULTURED PC12 CELLS AND SYMPATHETIC NEURONS DURING NEURITE OUTGROWTH. K. Kalil. M. Lee* and J. Rosenheimer. Dept. of Anatomy, Dept. of Physiology, and Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

In a previous report (Kalil and Pereda, J. Neurosci.8:4797-4808, 1988), we described a 33Kda membrane protein whose expression declined sharply during development of the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in developing the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in developing the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in developing the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in developing the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in developing the hamster CNS.

Supported by a grant from the Whitaker Foundation.

413.10  

The nerve growth cone is a highly motile and dynamic structure intimately involved in such diverse phenomena as neurite outgrowth, pathfinding, target cell recognition and synaptogenesis. Recent evidence has suggested that several environmental factors such as contact with other cell surfaces may be important for regulating growth cone motility. We have used time-lapse videomicroscopy to determine the effects of contacting contract with growth cones of sympathetic neurons from the rat superior cervical ganglion (SCG) and other cell surfaces, including other sympathetic growth cones and neurites. 45% of the contacts between growth cones of two sympathetic neurons resulted in the collapse of growth cone structure of one, but never both growth cones. Only 12% of the contacts between growth cones and neurites resulted in growth cone collapse. Contact with other cell surfaces, either red blood cells or fibroblasts never resulted in collapse. These studies suggest that the SCG may be composed of two subpopulations of neurons with different cell surface determinants, and that these determinants are more concentrated on the surface of growth cones than on neurites. Approximately half of the neurons of the SCG project rostrally, approximately half project caudally. Experiments are in progress to determine if these represent the two subpopulations that we detect in our culture system. Studies using the calcium indicator dye fura2 are also in progress to examine the mechanism of this collapse phenomenon.

(Supported by the McKnight Foundation and the PMA)
AN IMPROVED STAINING METHOD FOR SENILE PLAQUES AND NEO- 
FIBRILLARY TANGLES IN ALZHEIMER'S DISEASE: QUANTITATIVE 
COMPARATIVE IMMUNOHISTOCHEMISTRY TECHNIQUES. 
of Geneva, Switzerland, and Research Institute of Scripps Clinic, La Jolla, CA, USA.

In an attempt to better quantify the number and distribution of the neuropathological hallmarks of Alzheimer's disease we used a modified toluidine blue staining method to allow for the visualization of tangles and plaques in neurofibrillary tangles (NFT) and senile plaques (SP). This method can be applied to human brains collected after a short post mortem delay and fixed for immunoperoxidase labeling, as well as to brains kept in formalin for years or to paraffin-embedded materials. Sections were pretreated with 0.25% KMnO4 and then immersed in 1% K2S2O5 and 1% oxalic acid or a combination thereof, which were then reacted with 1% NaOH and 3 ml of 30% H2O2. Thioflavine was then diluted up to 0.0125% instead of 1%. This treatment removes lipids auto-
fluorescence. The method allows for the visualization of as much as 50% more SP and NFT as compared to the classical thioflavine S method or to argentaffin impregnations. Amyloid accumulation in the wall of small vessels was frequently observed. Extravascular amyloid deposits were also evident. A comparable method enhanced peptide immunostaining in human brains kept in formalin for 40 years. Finally, a very accurate automatic quantification of NFT and SP using a computer-assisted image analysis system was made possible by the heightened quality of the staining. This improved technique might be used to further analyze the regional and laminar distribution of the pathological lesions in senile and presenile cases of Alzheimer's disease.

CHROMATOGRAPHIC ANALYSIS BY HPLC OF ALZHEIMER'S DISEASE ASSOCIATED PROTEIN/S (ADAP) 
Barney E. Miller, PhD, Allen B. Simon, BS* and Hosseini A. Ghanbari, PhD* 
Mental Illness and Neurological Diseases Diagnostics, Abbott Laboratories, Abbott Park, IL 60064

The ADAP was purified -1250 fold by differential centrifugation, detergent extraction, and 2-dimensional PAGE analysis. Immunoelectrophoretic detection and visualization of the Alzheimer's disease brain tissue homogenate and analyzed by three HPLC methods. The methods were: gel permeation (Beckman Spherogel TSK 4000 PW, 2 peaks (the second peak was clearly multi-component). A single "sharp" peak of immunoreactivity was eluted from the affinity column. Fractions were analyzed by SDS-PAGE/Western Blots. The affinity purified material showed tripeptide bands of activity by this analysis. It is clear that ADAP is a mix of immunogenically similar proteins that may be iso-forms. Further work to purify and sequence ADAP is presently underway.

THE ISOLATION OF A RELATIVELY SPECIFIC PAIRED HELICAL FILAMENT ANTIBODY 
S.G. Greenberg and J. Schein.

We have isolated a monoclonal antibody (PHF-1) from mice injected with high concentrations of relatively nonaggregated populations of paired helical filaments (PHF). PHF-1 reacted with neurofibrillary tangles (NFT) and with neuritic processes. Double labeling of AD sections revealed that the staining of NFT and neuronal processes was present with PHF-1 at a level higher than Alz-50. However, unlike Alz 50, PHF-1 did not label neuronal normal tissue. Immunoelectron microscopy demonstrated that PHF-1 reacted with PHF in PHF-enriched preparations. Unlike other PHF antibodies, PHF-1 recognized the 57-68 kd PHF protein. The precipitation product was an unresolved higher molecular material present in PHF-enriched preparations. While it is difficult to detect PHF proteins in homogenates with PHF antibodies such as Alz 50, PHF-1 is unique in its ability to react with PHF proteins in both homogenates and cytoskeletal enriched preparations from AD cases. Weak or no reactivity was observed with normal cytoskeletal proteins, including purified human tau. Thus, PHF-1 appears to recognize a unique epitope present on PHF. While the PHF-1 epitope may result from either a unique sequence or a posttranslational modification of a normal cytoskeletal epitope, the relative sensitivity and specificity of PHF-1 indicates that this antibody will be useful for the specific detection of relatively larger classes of PHF proteins during different pathological conditions. This work was supported by NIH grant AG06803.
414.7
Neuronal mRNA levels are decreased in the neocortex of Alzheimer disease (AD) brains. Case to case variation in AD neuropathology may reflect stages of AD degeneration. In this study we asked at which stage the mRNA decreased. In these AD cases (60-91 yrs) and 13 controls (55-82 yrs) were selected. The AD cases had varying severity of neuropathological changes: neurofibrillary tangle (NFT) counts in the frontal cortex ranged from 0-55/mm² and neuritic plaques (NP) ranged from 5-94/mm². The frontal or parietal cortical was processed for quantitative Northern analysis with cDNAs for neurofibrillit light subunit (NF-L) and amyloid precursor protein (APP). Our results show an overall decrease of 40% for the NF-L and APP mRNAs in the AD cases. The NF-L mRNA inversely correlated with the number of NFT (r = -0.70, p<0.01), especially in the older cases (>70 yrs). The APP mRNA also had an inverse correlation with the number of NFT (r = -0.49) but this did not reach statistical significance. No correlation was seen between the numbers of NFs and NF-L or APP. We interpret these results to mean that in Alzheimer type, the decrease in neuronal mRNA in the cortex occurs with the appearance of NFTs.

414.8
In the brain of aged controls we studied the morphology of tangles in several cortical regions where these lesions appear first. We consistently found delicate Thioflavine-S positive threads, which contained a normal (NFT)-associated antigen and a few dysmorphic neurites surrounding a Thioflavine negative perikaryon full of lipofuscin. We also observed slightly thicker threads in the neuropil, some of which contained a normal perikaryon that had the appearance of early tangles. Next to mature tangles there were thick strongly fluorescent neurites, some of which were positive for cholinesterases. Adjacent sections stained for cholinesterases (AChE and Bohe) showed positive reaction in early and mature tangles and only in the thickest neurites. Similar images were observed in selected isocortical areas of Alzheimer's brains. We suggest that the sequence in the evolution of tangle formation may be as follows: 1) Amyloid starts to accumulate as delicate threads within proximal dendrites that are negative for cholinesterases. 2) Next, the perikaryon gets involved too. At this stage staining for AChE and Bohe parallels Thioflavine fluorescence. 3) Finally, mature old tangles appear to break into thick strongly fluorescent neurites, some of which are positive for cholinesterases. The beginning of AChE and Bohe accumulation may represent the threshold in the degeneration process when transport is interrupted, and these enzymes are trapped among the altered cytoskeleton. Supported by FISSE 88/922 and 89/635.

414.9
We and others (1) have previously reported evidence of chronic inflammation in Alzheimer brain tissue. This includes the presence of the complement regulatory molecule, CR1, on microglia, and CD54, and CD58 on lymphocytes. We now report positive staining of senile plaques, dystrophic neurites and some neurofibrillary tangles with antibodies to several components of the classical, not the alternative, complement pathway. Positive staining of these elements of Alzheimer brain tissue was obtained with polyclonal and monoclonal antibodies directed against C4d, C7 and a neoantigenic site on C5b-9, and a monoclonal antibody directed against the specific, alternative complement pathway factor, factor H. Positive staining was also obtained with mouse monoclonal antibodies directed against C4d, C7 and a neoantigenic site on C5b-9 and lymphocytes. These data suggest that microglial and lymphocytic infiltrates might be involved in the alternative pathway. Further studies are needed to identify the antigens involved in the staining of these elements of Alzheimer brain tissue.

414.10
ALTERATIONS IN LOCALIZATION OF CA²⁺ ATPASE IN ALZHEIMER DISEASE BRAIN. B. Sisken, W. Markesbery and T. Vanaman. Center of Neurochemistry, Departments of Anatomy and Neurochemistry, Pathology and Sanders Brown Center on Aging; Department of Biochemistry, University of Kentucky, Lexington, KY 40506.
Calcium metabolism alterations are of major interests in Alzheimer disease (AD) since abnormally high levels of internal free calcium are associated with neuronal degeneration. Significant changes in the localization classes of Ca²⁺-pumping ATPases which maintain low intercellular levels of calcium exist at the cell surface and in the internal membranes of the cell. We have localized in normal and AD hippocampus one distinct catalytic subunit of Ca²⁺-ATPase which we have previously identified by molecular cloning. In normal brain, astrocytes, oligodendrocytes, ependyma and microglia are non-reactive while ependymal cilia, microvilli, endothelial cells are reactive, hippocampal dentate granule neurons, CA4 neurons and ependyma, ependyma, CA3 neurons are highly reactive while CA2 and CA1 subiculum neurons are progressively less immunoreactive; the molecular pattern of the dentate gyrus is nonreactive. By contrast in AD, the proximal, middle and distal molecular layer of the dentate gyrus are reactive. CA4 neurons and ependyma are reactive at the thinnest neurites. CA3 clusters of synapses show pronounced immunoreactivity while CA4 through CA1 neurons resemble that in normals. Reactive astrocytes show increased reactivity to proper staining. Reactive microglia and CA3 reactive neurons and thin reactive neurites and reactive neurites are nonreactive. These findings suggest altered calcium metabolism in reactive astrocytes and ependymal. Immunoreactivity of CA³⁺ ATPase was observed in the CA2 and CA3 CA2 neurons and ependyma. These findings suggest altered calcium metabolism in reactive astrocytes and ependymal.

414.11
We examined possible translational differences between the Alzheimer and control mRNAs from postmortem brains. The focus was on the 5' cap structure of the mRNA since it is involved in the initiation step of protein synthesis. The translational activity of mRNA isolated from the control (n=7) and AD (n=7) cases was measured in the rabbit reticulocyte system in the presence and absence of the cap analogue 7methylguanosine 5' triphosphate (pppG). The absence of pppG control increased the incorporation of 35S methionine (1.6x10⁵ cpm/µg mRNA) as compared to AD mRNA (1.1x10⁵ cpm/µg mRNA). The same observation was made for the translation of mRNA in controls by 26% and in AD cases by 13.6% Mouse mRNA translated under identical conditions (2.8x10⁴ cpm/µg mRNA) showed a 45% increase in the presence of pppG. These results indicate that the AD mRNA may be relatively undercapped as compared to control mRNA which may result in the reduction in the protein synthetic activity of AD mRNA in vitro and in living patients afflicted with AD. Supported by ARAF.

414.12
Alzheimer's disease is characterized by the abnormal accumulation of several proteins in selectively vulnerable brain regions, including the hippocampus. Antibodies that recognize neurofibrillary tangle (NFT)-associated antigens (AAAs) and neurodegeneration in cultured rat hippocampal neurons. In untreated cultures SE2 antigen was expressed in most neurons with considerable variability in staining between neurons. Low to moderate levels of Alz-50 immunoreactivity were present in about 50% of the neurons. All neurons expressed low levels of ubiquitin. Subtoxic levels of glutamate or K⁺ increased the expression of SE2 and Alz-50 antigens but did not alter ubiquitin expression. Glutamate and K⁺ at levels that caused a progressive degeneration of neurons over a 24 hr period increased in the expression of all three AAAs, particularly Alz-50. There was a striking increase in ubiquitin levels only in degenerating neurons. Higher levels of glutamate which caused a 30% decrease in glutamate expression. CYCLOHEXIMIDE did not prevent induced increases in AAA levels suggesting that the increased levels of AAs might reflect the modification of existing proteins. Finally, we have found that treatments which increased gluatamate neurotoxicity did not express detectable levels of Alz-50 suggesting that Alz-50 antigen is a marker for neurons vulnerable to glutamate neurotoxicity. Collectively, these results suggest that the increased presence of at least some AAs can result from excitatory overactivity, and are consistent with a model in which normal proteins are modified by the activation of neuronal signal transduction systems. The data are also consistent with the involvement of glutamate in Alzheimer's neurodegeneration. (Supported by the French Foundation for Alzheimer's Disease).
GANGLIOSIDES PREVENT RECOGNITION OF BRAIN ANTIGENS BY SERUM ANTIBODIES FROM AGED MICE.

W. Worth, TX 76107-2690.

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We previously reported no detectable aluminum (Al) levels in seven AD and four control cases by X-ray microprobe analysis (Jacobs et al., Soc. Neurosci. Abstr., 12:944, 1986). Our data have since been replicated and extended by McGee and associates (McGee et al., Neurosci. Lett., 79:195, 1987). Crucial questions remain, however as to the exact molecular identity of the brain protein labelled by HLA-DR antibodies in normal elderly brain with HLA-DR immunoreactivity. MHC type II antigens such as HLA-DQ, -DP, and -DO are abundantly expressed in white matter of normal elderly brain, whereas the immunoreactivity for major histocompatibility complex (MHC) antigens in normal human and laboratory animals, we suggested, could be attributable to the expression of gangliosides upon IgG binding, brain cells were pre-incubated with monosialoganglioside (GM1) or mixed gangliosides (0, 1, 5, or 10 µg/10^6 cells) for 60 min, washed, and incubated with sera from old mice which had previously exhibited "brain-reactive" IgG3. When probed with peroxidase-conjugated, goat antimouse IgG, only the cells incubated with saline or 1 µg gangliosides showed immunoreactivity for major histocompatibility complex (MHC) antigens. The findings suggest that ganglioside treatments might protect CNS neurons from immunologic injury. (Supported by NIH grants AG06182 (MUF), AG07695 (HL), and NIH-BSRG grant RR05679.)

FUNCTIONAL CONSIDERATIONS OF NEUROIMMUNE MARKERS IN ALZHEIMER'S DISEASE. J. Rogers, S.D. Styrer, S.A. Allen, and W.H. Clevin. Institute for Biogerontology Research, Sun City, AZ 85351.

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Aluminum access to the brain: A possible role for transferrin. A. Jane Roskos and James R. Connor. Center for Neuroscience and Department of Anatomy, M.S. Hershey Medical Center, Hershey PA 17033.

Transferrin (TF), the iron mobilization protein from plasma utilizes a receptor on brain endothelial cells to transport iron across the blood-brain barrier. In addition to iron, TF binds aluminum. Indeed, as much as 30% of the TF in plasma is bound to aluminum. Two studies are described here: 1) Using 59Fe on brain membrane preparations we found that the 59Fe/TF receptor has a Kd of approximately 0.5nM; 2) We established that the TF-aluminum complex binds to and is internalized by oligodendrocytes. Some cells in the white matter which appeared to be astrocytes were immunoreactive in the AD tissue. Ferritin (ICN Immunobiologics, 1:300) immunoreactivity is specific to oligodendrocytes in normal brain tissue. Some ferritin immunoreactive cells are found in association with senile plaques and these cells may be macrophages or microglia. Quantitative analyses of TF and ferritin are in progress. The results of this study suggest that oligodendrocytes may play a role in iron regulation in the brain. Supported by Alzheimer's Disease Research, a program of the American Health Assistance Foundation.
414.19

**VISUALIZATION OF NON-MICROTUBULE TAU BINDING PROTEINS BY LIGAND BLOTTING**


Understanding the normal in vivo function(s) of tau and those properties of tau which may lead to abnormal aggregation states, would be enhanced by a knowledge of the full repertoire of proteins capable of interacting with this microtubule-associated protein (MAP). Towards this end, we have developed a ligand blotting assay to identify possible tau binding proteins. The procedure involves incubating nitrocellulose bound proteins with purified bovine tau followed by detection of bound tau with anti-tau mAb's. Incubation of rat cortex homogenates with 0.7 M HCl reveals high affinity binding to a triplet of proteins in the 100-150 kD range. These proteins are distinct from tau or tubulin dimers also seen in this range. Two of these proteins are enriched in preparations of taxol stabilized microtubules. Several protein bands are bound with lower observed affinity among these are tubulin, MAP1b, and MAP2. Work is underway to determine the prevalence of the high affinity tau binding proteins in human brain tissue of normal and Alzheimer's disease origin.

414.20

**IN VITRO STUDIES OF THE INTERACTION BETWEEN THE ALZHEIMER COMPONENTS α-ANTICYMOTRYPSIN AND β-PROTEIN**


The protease inhibitor α-antichymotrypsin (ACT) has been shown to be an integral component of the amyloid deposits of Alzheimer's disease and the similar deposits in Pick's disease and in normal aging in humans and monkeys. ACT was also found associated, together with the β-protein, in non-Alzheimer amyloid fibrils in sensorial, olfactory, arterial, and perivascular neuriyte plaques found in Holland (Abraham et al., submitted). Together these results indicate that there is a special association of ACT and the β-protein in the formation of amyloid. We are therefore examining the interaction between ACT and the β-protein in vitro. A radioiodine-labeled peptide corresponding to amino acids 1-28 of the β-protein becomes bound to ACT in an SDS-resistant complex. The use of cross-linking agents stabilizes even a greater amount of the peptide onto ACT. Other experiments suggest that the 1-38 peptide may bind to the ACT protein at an active protease-inhibitory site: (1) the addition of chymotrypsin to ACT prior to the addition of the peptide prevents the association and (2) the peptide prevents ACT from being able to subsequently inhibit chymotrypsin. An examination of the amino acid sequence of the β-protein suggests a region near the N-terminus, which shows striking homology to the active site of serine proteases. Studies with peptides having specific amino acids changed in this region are underway to test whether this protease-like region is the basis for the specific association of ACT and the β-protein. A model for the Alzheimer-like amyloid filaments based on this interaction will be presented.

414.21

**Polyepitope fragments derived from insoluble components purified from Alzheimer, Pick's and Parkinson's diseased cortex. G.D. Vogelzang, G.E. Dean*, and F.P. Zeman*. Dep. of Physiology, Molecular Genetics and Psychiary, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45276.**

Paired helical filaments (PHF) were electrophoretically purified from high density neurofibrillary tangle (NFT)-bearing Alzheimer (AD) parietal cortex. Straight filaments were isolated from identically prepared Pick's and Parkinson's brain tissue lacking NFT. Subsequent electrophoresis separated these relatively insoluble structures. Silver-stained protein profiles of these solubilized structures appeared identical on sodium doceyl-sulfate polyacrylamide gels. The predominant polyepitope fragments were limited to M₆₃-66 kilodaltons. Identically derived material from normal brain tissue lacking NFT's had a similar protein profile, but only amorphous aggregates were observed in the material solublized. PHF and straight filaments were equally insoluble, but straight filaments had a smaller diameter (<10nm) and lacked the helical nature of PHF. Only the AD derived material had demonstrated PHF-specific immunoreactivity as judged by Western blot analysis. Actin immunoreactivity was indicated at the M₆₆-64-66 kilodalton range. The other brain types also displayed a reduced amount of this unusual actin-immunoreactivity/µg protein at the exact molecular weight range. It appears that the proteins associated with PHF structure may be common to all brain types and the inherent insoluble components that can be derived from each, regardless of their ultra-structure. The feature which causes AD-specific immunoreactivity may be attributed to a conjugation or modification of these common proteins.

414.22

**SURCELLULAR THREE-DIMENSIONAL ANALYSIS OF HUMAN CORTICAL PYRAMIDAL NEURONS WITH ALZHEIMER PATHOLOGY BY HIGH VOLTAGE ELECTRON MICROSCOPY AND COMPUTER-AIDED RECONSTRUCTIONS FROM SERIAL SECTIONS. S. J. Young, D. Hessler*, T.J. Deerinck, and M.H. Ellisman (SPON: L.L.Lugasi, Lab for Neuroelectronics, U.C.S.D., Mesa, CA).**

We have been studying alterations in the form, content and spatial distribution of subcellular elements of cortical pyramidal neurons obtained from human biopsy specimens exhibiting the pathological disease. We have compared neurons differing in pathology, as indexed by the amount of paired helical filaments present. Differences between brain regions were examined to identify components that would allow three-dimensional reconstruction in micrographs from serial sections. Computer assisted three-dimensional reconstruction of electron micrographs from serial sections, and (2) stereo-pair views of thic sectioned tissue obtained with the high voltage electron microscope. We have utilized serial reconstructions of normal and disease (AD) tissues, and from normal and disease (AD) tissues.

414.23

**GROWTH RELATED PROTEINS IN ALZHEIMER'S DISEASE. E. Mastaja*, R. Terry*, T. Saltel, (sponsored by R. Katzman). University of California, San Diego, School of Medicine, Dept. of Neurosciences, M-024, La Jolla, CA 92093, U.S.A.**

Some theories explain the pathogenesis of Alzheimer's disease (AD) as the result of a decrease in the production of growth factors (GF). Other studies, however, suggest a sprouting reaction in AD possibly in relation to compensatory involvement of GF's, we performed immunohistochemical studies of tissues which showed an increased in GF stimulation, (eg: fos, PKC). We found, by comparing five AD and five control cases, a 40% decrease in the number of immunostained neurons in the AD nucleus basalis with anti-fos and anti-PKC α, β and γ. In contrast, the neocortex and hippocampus of AD cases presented an increased in the number of immunostained neurons with anti-fos as well as immunolabeled neurites in the plaques with anti-PKC α, β and γ. These data suggest a possible decrease of GF's stimulation in subcortical areas, probably related to the hippocampal deafferentation with an increase in the GF stimulation in the hippocampus and neocortex possibly in relation to compensatory sprouting reaction.

414.24

**EPIDERMAL GROWTH FACTOR EXPRESSION IN BRAIN TISSUE OF ALZHEIMER'S AND OTHER NEUROLOGICALLY DEMENTED PATIENTS. S. D. Stover, S. Allen and J. Rogers. J.R. Roberts, Center, Institute for Biogerontology Research Sun City, AZ.**

Epidermal growth factor receptor (EGFR) is a 170 KD integral membrane protein. It contains a tyrosine kinase moiety, and is activated by the binding of epidermal growth factor, transforming growth factor and vaccina virus growth factor. EGFR binding results in increased mitotic activity and upregulation of cytoplasmic signaling pathways within the target cell. We have utilized the EGFR immunohistochemistry as well as Western blot analysis to examine EGFR expression in the brains of non-demented and clinically demented patients (Alzheimer's disease, Parkinson's disease, multi-infarct dementia). Immunohistochemical analysis of EGFR distribution within the brain demonstrates a relationship between presence of dementia and the expression of EGFR. Ten patients with a variety of neurological dementia were positive for EGFR expression throughout all regions of brain examined. Eight patients without dementia did not exhibit any EGFR immunoreactivity. One patient without clinical diagnosis of dementia and one with Parkinson's disease were negative for EGFR. Our preliminary results indicate that the EGFR expression (1) is associated with the presence of clinical dementia, (2) is present throughout the brain once induced, (3) is predominantly localized to the blood vascular endothelial cells of affected patients, and (4) may be inducible by factors common to physiological states which induce dementia. Supported by NIA AGO 7367-01 (JR).

Antiser against specific regions of the Alzheimer [amyloid protein precursor (APP)] was used to study the effects of nerve and epithelial growth factors on the expression and processing of this protein in PC12 cell cultures. Two major APP proteins containing the Kunitz-type protease inhibitor (KPI) were observed in control and in PC12 cell cultures. One major APP was identified as an Mr of 140,000 and the other as an Mr of 150,000. Addition of nerve growth factor (NGF) to PC12 cells resulted in a large decrease in the amount of the 150,000-kDa protein and an increase in the amount of the 140,000-kDa protein. No detectable change was seen in the levels of APP mRNA. Medium from naive PC12 cell cultures contained minimal amounts of either the 145,000- or the 100,000-kDa APP. These data suggest that the KPI-containing APP may be modulated by intercellular contact.


We have developed and produced 6 new monoclonal antibodies (MAb's) that selectively bind to a group of proteins (isoforms) which appear as 3 major bands (53, 61, and 67 kDalloons) on SDS-PAGE (20% acrylamide) using ALS25 monoclonal antibody. These proteins, referred to as ADAP, are present in Alzheimer Disease (AD) and some Down Syndrome brain tissue and absent in normal brain. These ADAP proteins are present in normal neocortical area 7, but are more prominent in areas associated with the corticocortical systems, as reflected by a differential screening of a mixed Alzheimer's/Control hippocampal cDNA library. Nucleotide sequencing of ADAP-1 identified an open reading frame of 449 amino acids, coding for a putative 52 kDa protein. To confirm this reading frame, cDNA gapped was transcribed into vitro and translated using rabbit reticulocyte lysates. A prominent 52 kDa protein was observed following translation indicating an authentic open reading frame of the appropriate size. The nucleotide and deduced amino acid sequence of this protein show 78-80% identity to rat sulfated glycoprotein-2, the major secretory product of Sertoli cells in the rat testes. A consensus signal peptide, 6 presumptive ASN-linked glycosylation sites, 10 CYS residues and a proteolytic cleavage site are conserved between these two proteins. These data suggest that ADAP-9 may encode a highly processed glycoprotein related if not identical to a human analogue for rat sulfated glycoprotein-2. This function in brain is unknown. Supported by AMRC Grant # A005142, the John D. and Catherine T. MacArthur Foundation Research On Successftul Aging(CEF) and ARRA Grant 1180-88-069 (PM).


A quantitative neuropathological analysis was performed in a subpopulation of the Alzheimer’s Disease (AD) patients that presented a visul defect referred to as Balinťs syndrome (ADB) early in the course of the disease. Balinťs syndrome is a defect in visuospatial processing and is evident in AD. In area 17, Meynert cell counts in area 17 were significantly lower (41%) in ADB. These data suggest that in some cases of AD, specific neurologic symptomatology may be caused by the selective loss of specific corticocortical systems, as reflected by a differential distribution of the neocortical markers of the disease. Supported by grants from NIA (AG06647) and FNRX (83.495.087).

ALZHEIMER’S DISEASE: NEUROPATHOLOGY THURSDAY AM


Clone ADPC-9 encodes a 2K RNA which is overexpressed in Alzheimer’s disease hippocampus. ADPC-9 hybridizes by differential screening of a mixed Alzheimer’s/Control hippocampal cDNA library. Nucleotide sequencing of ADPC-9 identified an open reading frame of 449 amino acids coding for a putative 52 kDa protein. To confirm this reading frame, cDNA gapped was transcribed in vitro and translated using rabbit reticulocyte lysates. A prominent 52 kDa protein was observed following translation indicating an authentic open reading frame of the appropriate size. The nucleotide and deduced amino acid sequence of this protein show 75-80% identity to rat sulfated glycoprotein-2, the major secretory product of Sertoli cells in the rat testes. A consensus signal peptide, 6 presumptive ASN-linked glycosylation sites, 10 CYS residues and a proteolytic cleavage site are conserved between these two proteins. These data suggest that ADPC-9 may encode a highly processed glycoprotein related if not identical to a human analogue for rat sulfated glycoprotein-2. Its function in brain is unknown. Supported by AMRC Grant # A005142, the John D. and Catherine T. MacArthur Foundation Research On Successful Aging(CEF) and ARRA Grant 1180-88-069 (PM).


To test this theory, we grew embryonic rat hippocampal neurons in a serum-free culture medium that supports normal cultures of neurons but not glial processes. This medium contained 10% human serum. After 24 hr., neurons were fixed, one of three AD-specific stains was applied, and digitized fluorescence intensities were recorded. This method binds to the ß-amyloid found in AD plaques. In culture, 10 of 12 sera tested produced significantly more fluorescence than untreated neurons. Fluorescence was particularly strong among clusters of cells. 2) Alz-50 obtained from P. Davies is a monoclonal antibody (mAb) generated by immunization with an AD brain in which both neurons and astrocytes were stained. This mAb binds to the ß-amyloid found in AD plaques retrieved from KI & Wisniewski produced immunofluorescence in neurons treated with sera as follows: one AD serum >> one normal serum >> no serum. These results show that a serum factor can induce ß-amyloid neuropathology, which in the AD brain may be aided by a compromised blood-brain barrier. Funded by ONH and the Pearson Foundation.


Previous studies of dendritic extent in normal aging have led to the suggestion that, contrary to long accepted dogma capable of a remarkable degree of plausibility. Evident has accumulated that alteration or loss of this latent plastic capacity may be a pathophysiologic feature of Alzheimer’s disease (AD). In the present study, dendritic extent was measured in human basal forebrain in normal aging and AD. From over 60 cases obtained at autopsy through the Rochester Alzheimer’s Disease Project (RADP), 12 subjects were assigned to each of the following two groups (6 per group): 1) normal aged adults (mean 81.7 years, range 79-84 years), and 2) AD (mean 81.8 years, range 78-84 years). All cases were examined by a panel of neuropathologists to have the diagnosis of Alzheimer’s disease (AD). In the present study, dendritic extent was measured in human basal forebrain in normal aging and AD.

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The deposits occurred in three forms: 1) large, translucent, spherical encountered; these were quite variable with respect to the size and number of neurons. In long term survival experiments (up to 11 months post-lesion), we frequently found conspicuous mineralized deposits in the globus pallidus (GP) resembling neurons; and 3) as a flocculant accumulation of material surrounding placement of the BFC lesion do not precisely coincide. Typically, the deposits 2) smaller, more irregular to spheroidal, often sometimes with a cap, resembling neurons; and 3) as a flocculent accumulation of material surrounding the injection site where BFC cell loss is most sparing of BFC neurons. Thus, it is unclear how these mineral deposits relate to correlates during the course of AD.

MRI appears to be a sensitive technique to quantify neural degeneration and functional deficits (e.g. Greenland, B. Am. J. Psychiatry, 1988). We have examined basal cortical levels in Alzheimer patients (n=6) and same-aged, healthy controls in 12 patients with hippocampal atrophy at 0700. Samples were taken every hour for 25 h and cortisol levels were estimated using a radioimmunoassay (Krey et al., Endocrinology, 1975). Plasma cortisol levels were significantly increased in Alzheimer patients during both the AM (9.6±1.1 vs. 5.9±0.3 µg/dl) and PM (7.2±0.7 vs. 8.7±0.4 µg/dl) phases of the cycle. The differences occurred during the mid to late AM and early PM time points. The two populations exhibited virtually identical curves for cortisol over the diurnal cycle, with peak cortisol levels achieved at about 0800-0900h. Preliminary data indicate a similar trend for ACTH.

These data are consistent with previous reports and suggest that there is an increase in basal HPA activity in Alzheimer patients.


Heat shock proteins (HSPs) are ubiquitous polypeptides expressed in response to a wide variety of cellular stressors. To investigate the changes of expression of these proteins during Alzheimer's Disease (AD), we examined medial temporal lobes of AD patients and age-matched controls. Utilizing immunocytochemical techniques and monoclonal antibodies to two HSPs of M.W. 78Kd (D. Bole, Yale Univ.) and 72Kd (W. Welch, UCSF), we found that in control brains, HSP72 was present uniformly in neurons, glia, and capillaries, whereas HSP72 antibodies stained neurons in the subiculum and CA1 intensely, and neurons and glia in the dentate gyrus, other hippocampal fields, the entorhinal cortex, and the cerebral spinal fluid. Western blots confirmed the identification of these HSPs.

In AD brains, the pattern changed dramatically. HSP72 antibodies intensely stained neuritic plaques and neurofibrillary tangles. HSP72 was found only in cytoplasmic normal neurons, and even increased in neurons in the entorhinal cortex and CA3. Double labeling with Aβ-antibodies (P. Davies, Albert Einstein College of Med.), which stain plaques and tangles, showed that HSP72 did not colocalize with pathologically affected neurons.

These findings suggest that HSPs may undergo changes in expression during AD and, therefore, may be useful molecular tools in deciphering the underlying pathogenesis of AD.

SELECTIVE LOSS OF CENTRAL PATHWAYS FOLLOWING VIRAL INOCULATION IN THE RAT Olfactory System. J.H. McLean, M.T. Shipley and D.J. Bernstein*. Memorial Univ. of Newfoundland, St. John's, NF, Canada, A1B 3X6, Univ. of Cincinnati College of Medicine, Cincinnati, OH, 45267-052 and The Gamble Institute, Cincinnati, OH.

We previously examined the possibility that the olfactory system serves as a portal for environmental agents that may cause Alzheimer's disease (AD). We determined that the transeptal spread of a virus in the rat olfactory system correlates well with the regions known to degenerate in AD (McLean et al. '87). In this study, the short and long term effects of viral transport and replication in olfactory pathways were determined in order to correlate these effects with the known neurochemical changes observed in AD.

In anesthetized rats, 100 nl of 10^6 PFU/ml HSV1 (F strain) was injected into the olfactory bulb. Following survival of 3-5 days, rats were sacrificed by perfusion with 4% paraformaldehyde. Brains were processed by immunocytochemistry to visualize neurotransmitter or virus localization. Following these short survival periods, virus-infected cells could still express neurotransmitter in cholinergic neurons of the diagonal band, serotonergic neurons in the raphe nucleus, and noradrenergic neurons in the locus coeruleus. These results compare well to the deficit seen in AD, an observation of particular importance are apparent. The present results may provide insights into the etiology of AD. Supported by the American Health Assistance Foundation, NIH NS 23348 and NIAID AI-23482.

The presubiculum is a significant destination for fiber projections from the anterior thalamic nuclei. In turn, fibers from this area project to the entorhinal cortex to close the limbic circuit originally described by Papez. Immuno-staining for β-amyloid protein or modified Bielschowsky’s silver staining has revealed the occurrence of diffuse amyloid deposits in the presubiculum in all Alzheimer cases so far examined in vivo. Observations on serial blocks showed these deposits to be localized in the presubicular papyramidal layer of the presubiculum proper, the transubiculum and the parasubiculum, while the parasubiculum was devoid of such deposits. Bielschowsky’s staining indicated that the amyloid deposits preceded the appearance of neurofibrillary tangles and dystrophic neuritis in this region. The aggregated microglial reaction which was characteristically associated with the classic senile plaques was absent, since the presubiculum also receives afferents from frontal and parietal association cortices, the changes in this area in Alzheimer’s disease could contribute to isolation of the hippocampal formation from other cortical areas. Furthermore, coexistence of cortical inputs might play a significant role in the formation of diffuse amyloid deposits in the presubiculum.


Alzheimer disease (AD) is associated with loss of neurons in numerous cortical and subcortical regions of the human brain. The anterior thalamic nuclei (ATN) achieve unusually high counts of senile plaques and neurofibrillary tangles in AD, and has been reported to show a reduction in volumetric cell density. Quantitative data on cells from the anterior thalamic nuclei, an important criterion for distinguishing neurons from glia, are lacking.

Six neuropathologically confirmed AD specimens and six age-matched control cases were selected. Fifteen micron thick coronal sections were prepared from formalin fixed, paraffin embedded tissue and stained with cresyl violet. For each case, 30 to 80 fields from the cortical and magnocellular basal nuclei each were analyzed, utilizing extensive on-line editing with a Kontron IBA image analysis.

Significant shifts in the size distribution of cells of the cortical and basal nuclei were evident. There was reduction in volume density of the largest neurons and an increase in number of intermediate sized cells in the AD cases. Overall volumetric cell density was shown to increase in the basal but not cortical nucleus in AD. C3 cells, a cell type showing significant loss of volume of these nuclei in AD, it is nonetheless evident that a true loss of cells occurs in the amygdala.


The appearance of brain-reactive antibodies (BRA) and behavioral impairments occur at relatively young ages in autoimmune-prone NZB/BINJ mice when compared with C57BL/6N mice, a non-autoimmune strain. As each age, the serum BRA increases along with gain. Is the net result an increase or a decrease in resolution? On the costs side, energy is required for axonal and synaptic transmission. In turn, fiber projections from frontal and parietal association cortices, the changes in this area in Alzheimer’s disease could contribute to isolation of the hippocampal formation from other cortical areas. Furthermore, coexistence of cortical inputs might play a significant role in the formation of diffuse amyloid deposits in the presubiculum.


Although a well-known dogma of neurobiology holds that adult brain cells are post-mitotic and therefore not capable of proliferating, we have attempted to maintain in culture cells from different brain regions of aged, non-Autopsy brains of Alzheimer’s, Parkinson’s and non pathological conditions. Different culture conditions have been evaluated and optimized for approximately fifteen different regions from brain autopsies of 12 Alzheimer’s cases, 4 Parkinson’s cases, and 4 control cases. Under optimal conditions, a small percentage of brain cells (0.01-0.001%) can be maintained in vitro in viable condition for variable periods of time. Although we are not investigating the fate of these cells, their neuronal or at least non-glia nature is suggested by the fact that (1) glial cell markers do not stain and (2) ciliated and proliferate under our culture conditions. Cultured brain cells do not stain with endogenous or gli specific antibodies (e.g., Factor VIII, GFAPI). (3) cultured brain cells are stained by the anti-neurant antibody SMI-32 and also, in a smaller percentage of cells, with antibodies against phosphorylated neurofilaments. Furthermore, some of these brain-derived cells appear to divide under our culture conditions, as shown by increase of cell numbers and thymidine incorporation.

We conclude that cells from adult brains can be maintained in culture for longer times than previously thought. Although further studies are required for their characterization, cultured cells may provide an interesting model for studies of CNS cells in both normal and pathological conditions.
415.3


Trains of pulses of 1-1.5 s duration, varying in rate from 1-100 Hz, were delivered to single motor axons of the median nerve by the method described previously (Westling et al. Neurosci. Lett. 14, 1988). Stimulus rates were delivered alternately in both ascending and descending order. Force responses of individual motor units were recorded and the pairs of maximal forces were calculated and used to calculate the magnitude and direction of vector summed forces. EMG was recorded from both proximal and distal muscle surfaces.

Conventional sigmoid force/frequency curves were generated for all units, with minimal twitch fusion evident at 8-10 Hz, 50% maximum force at 15 Hz, and maximal force at 50-100 Hz. The rates giving 50% maximum force were influenced by the sequence of stimulus pattern delivery and by the unit's previous activation history, but were not well correlated with twitch contraction or 1/2 relaxation times. When stimulating at 1-0 Hz EMG potentials remained constant throughout each pulse train, but during 100 Hz EMG amplitudes declined rapidly, despite a well maintained force plateau.

Supported by USPHS grants NS 14756 & HL 30062, and by the Medical Research Council of Sweden.

415.5

EXTRACELLULAR RECORDING OF RHYTHMIC SPONTANEOUS DISCHARGE IN UNTREATED AND DRUG-TREATED SYMPATHETIC GANGLIA. K. A. Alkadhi and V. H. Hogg*.* Department of Pharmacology, University of Houston, Houston, TX 77204-5515.

It is well known that mammalian enteric ganglia possess an integrative system for rhythmic discharge analogous to that of the vertebrate central nervous system. Sympathetic ganglia, on the other hand, have been traditionally considered as simple relay mechanisms with no such integrative function. However, observations made in isolated superior cervical ganglia (SCG) of rabbits and rats suggest that this ganglion can, under certain conditions, fire ongoing rhythmic discharge which triggered the rhythmic firing. Chronic pretreatment of ganglia with no such integrative function. However, observations made in isolated superior cervical ganglia (SCG) of rabbits and rats suggest that this ganglion can, under certain conditions, fire ongoing rhythmic discharge which triggered the rhythmic firing. Chronic pretreatment of ganglia with putative catecholaminergic SIF interneurons are not involved in the generation of rhythm and that sustained transmitter release by any procedure could trigger the rhythmic discharge. This generation, re-emphasising a central role for descending sensory interneurons in the presence of strychnine. Strychnine application and could contribute to rhythm generation, re-emphasising a central role for descending sensory interneurons.

Supported by USPHS grant NS 14756 and the Medical Research Council of Sweden.

415.4


The minimum excitation rates required for optimum force generation were investigated in 21 single human motor units of the thenar muscles by stimulation of the median nerve above the elbow as described previously (Westling et al. Neurosci. Lett. 14, 1988). First, two pulses were delivered 500 ms apart; then repeatedly at intervals reduced to 5 ms. The interval generating maximum twitch summation was then repeated as a third pulse was again delivered at progressively shorter intervals. This process was repeated until 10 was established; a procedure similar to that used for cat motor units by Burke et al. (Brain Res. 109: 515, 1976). Most human units generated maximum force in pulse trains where the first 2 shocks were delivered 5-10 ms apart, followed by longer intervals. These rates were compared to those seen in human voluntary contractions.

415.6

AGE RELATED DIFFERENCES IN SPONTANEOUS AND NMDA EVOKED SPINAL MOTOR PATTERNS: AN IN VITRO STUDY OF NEONATAL MICE. P. Hernandez*, R. Elbert* and W.H. Droges. Dept. of Biology, Texas Woman's University, Denton, TX 76204.

The objective of this study was to characterize the motor pattern generating capability of spinal cord-hindlimb explants taken from Balb/C mice aged birth to 4 days. Spontaneous and NMDA evoked EMG activity from the gastrocnemius (G) and tibialis anterior (TA) muscles were recorded and bursting sequences exhibiting the same timing as intact locomotion were included for analysis. Spontaneous locomotor rhythm occurred in only 4 of 76 experiments and those cases involved explants of age of 4 days. Separately, explants were tested with and without: 1) hemisection of the spinal cord; and 2) magnesium (1.0 mM) present in the perfusion solution. Of all combinations, nonhemisected explants perfused with magnesium free artificial CSF were most responsive to bath application of 1 - 5 μM NMDA. Using that preparation, motor rhythm was most often observed in 2 day old mice. The threshold dosage for evoking rhythm tended to be lower (1.5 - 2.0 μM) for animals ≤ 2 days of age. The EMC observed in vitro was often an alternation of entire bursting sequences rather than a cycle-to-cycle phasing. (NIH Grant # 1 R29 NS 25250-01.)

415.8

RESPIRATORY NEURON POPULATIONS IN THE VENTROLATERAL MEDULLA OF THE IN VITRO NEONATAL RAT BRAINSTEM-SPINAL CORD PREPARATION. John J. Greer, Jeffrey C. Smith & Jack L. Feldman. Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

An in vitro preparation of the isolated brainstem-spinal cord of the neonatal rat is presently being used in our laboratory to study cellular and synaptic mechanisms underlying the neurally generated respiratory neuronal populations in vivo. Previous studies utilizing this preparation have suggested that neuron populations in the ventrolateral medullary region of the neonatal cat mediate respiratory rhythm and pattern formation (Smith & Feldman, Soc. Neurosci. Abst. 14, 1988). We have now conducted detailed maps of the spatiotemporal patterns of neuronal activity in these areas. Extracellular recordings of neurons in the vicinity of nucleus ambiguous from the level of the caudal facial nucleus to areas caudal to the obex were made with dye-filled glass microelectrodes (2% pontamine sky blue in 0.5 M NaAc solution; impedance=7-12 MΩ). We identified and mapped five major respiratory cell types with distinct temporal discharge patterns: inspiratory (I), pre-inspiratory discharge starting in I (i phase), pre-inspiratory discharge starting in E phase ((E) phase), tonic E, late E, and biphasic E cells (on of phase (i phase)). Each of these cell types was found throughout the ventrolateral medullary region with a relatively high concentration of tonic and late E phase neurons at the level of rostral nucleus ambiguous. This distribution resembles the spatial distribution of respiratory neurons in the ventral respiratory group of the medulla in vivo. These results demonstrate the presence of a complex spatiotemporal pattern of neuronal activity in the rat medulla in vitro at a level where the first wave of respiratory neuronal activity. These studies provide the necessary groundwork for further analyses of the neuronal mechanisms underlying respiratory rhythm and pattern generation in the rat in vitro system. Supported by NIH Grants HL 45959 and HL 02054.

A 448-channel optical monitoring apparatus, using a round-shaped semi-intact abdominal ganglion preparation with siphon and large number of neurons. The 128-channel optical recording circuitry and pattern generation: invertebrates and models.


We initiated a series of anatomical studies to elucidate the neuronal circuitry underlying the generation of rhythmic jaw movements. Initial experiments using iontophoretic injections of WGA-HRP in the trigeminal motor nucleus identified trigeminal pre-motoneurons (Trig-PMs). Trig-PMs were found in the mesencephalic nucleus of V (Mes 5) ipsilaterally, principal sensory nucleus of V (Pv5) bilaterally, spinal trigeminal tract (Sp5) bilaterally, intertrigeminal region ipsilaterally, and bilaterally in the parvicellular reticular formation extending from the hypoglossal nucleus to the caudal aspect of the trigeminal region. No labeling was found in the large cells of the caudal gigantocellular reticular nuclei. Previous physiological experiments from our laboratory have indicated an excitatory amino acid as a neurotransmitter that mediates reflexes and cortically induced jaw movement. Therefore, we performed a series of glutamate mapping studies to determine which brainstem regions involved in jaw movements were also glutamatergic. Following a transcardiac perfusion of 5% carbodiimide and 5% glutaraldehyde, sections of the brainstem region were incubated for 24 hours in a monoclonal antibody directed against glutamate. The ABC immunohistochemical reaction was utilized. Glutamatergic cells were found in the inferior olive, solitary nucleus, external cuneate nucleus, prepositus hypoglossal nucleus, Sp5, Sp5, vestibular nucleus, ventral cochlear nucleus, superior olive, Mes 5, parabrachial nuclei, central nucleus of the inferior colliculus and posterior reticular formation including nucleus gigantocellularis, paragigantocellularis and paraventricularis. Currently we are performing a double labeling study combining the retrograde tracer, gold bound WGA-HRP, with glutamate immunohistochemistry to determine the locations of specific glutamatergic trigeminal pre-motoneurons. Funded by NIH-NIDCR grants DE 06763 and DE 06193.

CENTRAL OESOPHAGEAL CONTROL IN THE RAT - IS IT BILATERAL? D. Nieser and Y-T. Wang* (Sponsor: P. Redfern), Faculty of Medicine, Memorial University of Newfoundland, St. John's, N.L, Canada A1B 3V6.

The hypothesis that central control for the oesophageal peristalsis is intrinsically bilateral was investigated. In rats anaesthetised with urethane, deglutitive and cholinesterase-mediated oesophageal peristalsis were produced by application of bicuculline methiodide and muscimol, respectively, to the surface of the nucleus of tractus solitarius (NTS; 50-100 pmol, 0.1 µl). 1. Topical pretreatment with muscimol (5-10 pmol, 0.1 µl) abolished, but not totally, deglutitive-evoked peristalsis. 2. Ipsilateral acute or subchronic vagotomy resulted in failure of distal and decrease in cervical peristalsis. 2. Ipsilateral acute or subchronic cervical vagotomy resulted in failure to distal and cervical peristalsis. 2. Ipsilateral acute or subchronic cervical vagotomy resulted in failure of distal and decrease in cervical peristalsis.

To improve the spatial resolution of the optical method described, we plan to construct a family of variations on an approximate string of commands, and then judge each of these candidate strings against memories of what strings succeeded in the past for similar-but-not-identical conditions. Thus one needs an array of planning modules that may be even better than their parent string, have an even better fit to emerging memories of past successes and failures, and can adapt even during "live acts," a near-uniform choral-like population of strings may emerge. I have called this a Darwin Machine (Nature 350:33-34, 1987).

CIRCUITRY AND PATTERN GENERATION: INVERTEBRATES AND MODELS.

415.9


The physiological identification of glutamatergic trigeminal interneurons by antidromic stimulation of their terminals is usually difficult because of short conduction distances. One is left with the option of classifying interneurons by ejection of first order afferents, motoneurons and sensory neurons. In the work described, we were able to identify groups of interneurons terminating in the contralateral trigeminal (Mot. V) which were located in the interstriaiglinal area and the most anterior portion of subnucleus oralis (Landgren et al., Exp. Neurol., 66: 98; 1981). It was possible to record from the Mot. V and record antidromic spikes on the contralateral side.

Using standard criteria for antidromic identification, 37 neurons were identified as conjunctival interneurons. Their conduction latency range was 0.4 to 2.0 ms and conduction velocities 3.5 to 17.6 m/s. 14 units had intraoral, 4 had peri-oral, 2 had both intra- and peri-oral receptive fields. Dorsal receptive fields for the rest of the units were not determined. In addition, many of these neurons were excited at short latency (<5 ms) by stimulation of the contralateral saccus motor cortex. The discharge pattern of eight of these units was phasically modulated during fictive mastication. The role of these neurons in generating the pattern of mastication and the jaw opening reflex is under investigation. Supported by the Canadian MRC.

415.10


A possible preadaptation for the neural machinery underlying language and thought can be seen in that required for planning sequential movements. If it isn’t a standardized movement sequence such as the sequence one has to generate a family of variations on an approximate string of commands, and then judge each of those candidate strings against memories of what strings succeeded in the past for similar-but-not-identical conditions. Thus one needs an array of planning modules that may be even better than their parent string, have an even better fit to emerging memories of past successes and failures, and can adapt even during "live acts," a near-uniform choral-like population of strings may emerge. I have called this a Darwin Machine (Nature 350:33-34, 1987).

Since hand-arm sequencing circuitry in the brain has a strong overlap with what language circuitry is located in left brain, maybe the same Darwin Machine can do a double-duty for language and planning ahead. Instead of sequencing hand-arm commands and grading each candidate train by memories of previous successful throws, suppose that a track planned a tongue-tip sequence and graded each such candidate train by the rates of yawns and by the individual’s memories of similar verbal situations. The massively-serial buffers’ shaping-up process, from crude variation to a near-uniform population, is strikingly similar to the immune response shaping-up antibiotics to fit an invader, or biological evolution shaping a species’ bodystyle and behavior to fit a new environmental niche. Thus the well-formed sentence, and the reliable plan of action, have some strong analogies to familiar selection: evolutionary phenomena such as the Baldwin Effect and Gause’s Principle may prove useful analogies in understanding language and consciousness.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

THURSDAY AM
KINETIC ANALYSIS OF MEMBRANE CURRENTS IN THE HEART INTERNEURONS OF THE MEDICINAL LEECH. T.W. Simons, J.D. Angstede, R.I. Calabrese. Dept. of Biology, 1555 Pierce Drive, Emory University, Atlanta, GA 30322

A number of endogenous membrane currents contribute to the oscillatory properties of the heart interneurons (HN) of the leech. The initial step in modeling this network as a half-center oscillator is to develop kinetic descriptions of the currents based on voltage clamp and whole cell clamp recordings.

We have developed this method of analysis for three voltage-dependent conductances, that is, currents responsible for 5's and steady state conductances by using measured rate constants for state transitions of these conductances. These rate constants are exponential functions of membrane potential (Hodgkin & Huxley, 1952, J. Physiol. 117-540). We were able to model I_h, a hyperpolarization-activated inward current, which contributes to recovery from inhibition (Angstede & Calabrese, 1989). The rate constants were calculated from the decay phase of the delayed rectifier current. We modeled an I_c, an I_v, and a slow component (r = 100-300 ms).

A preliminary model of the graded synaptic current underlying inhibition between HN cells was created from the voltage response of a post synaptic cell to a presynaptic voltage step. The synaptic current consisted of a component dependent and a component independent portion, both of which were functions of synaptic voltage.

The kinetic models of these four currents will form the basis for a half-center oscillator model of two coupled heart interneurons.

ACTIVATION OF THE GASTRIC RHYTHM OF THE CRAB STOMATOGASTRIC GANGLION BY SDNRFLamide. J.M. Weisberg and E. Marder. Biology Department, Brandeis Univ., Waltham, MA 02254

In the crab, Cancer borealis, the gastric rhythm of the stomatogastric ganglion (STG) is highly spontaneously active in control saline, even when the STG is left attached to neural inputs from the anterior Commissural (CoG) and Oesophageal (OG) Ganglia. We have applied a variety of substances to the STG to modulate, known to be present in inputs to the STG, to find a substance that will activate the gastric rhythm. We now find an extended FMRFamide-like peptide SDNRFLamide, originally purified from Homarus americanus (Trimner et al., J Comp. Physiol. 266: 16-26, 1987) either applied alone (10-6 to 10-2 M) or in conjunction with the mammalian gastrin, polecarpine (10-4 to 10-3 M), reliably initiates robust gastric activity. Both polecarpine and SDNRFLamide also strongly activate the pyloric rhythm. Since many of the neurons of the STG can fire in both pyloric or gastric time, these substances allow us to explore the mechanisms by which neurons are shifted from one motor pattern to another by modulators. Supported by NS17813.

THE ROLE OF THE BRANCHIOSTEGITE NERVE IN CRAB GILL VENTILATION. K.P. Rajabekhah and J.L. Winkens. Dept. of Biological Sciences, University of Calgary, Calgary T2N 1N4, Canada

The branchiopterite nerves (BN) originate from the dorsal-lateral surface of the thoracic ganglion posterior to the scaphognathite nerves and innervate the walls of the branchial chambers. The sensory components include mechanoreceptors and other as yet unidentified receptors. Stimulation of the BN modifies the ventilatory rhythm in several ways including changes in pumping rate, switch from forward to reverse pumping, and in some cases complete reversals when branchial pressure becomes positive. Artificially raising branchial pressure silences, while lowering the pressure increases ABEM spike frequency. The ABEM spike frequency. Thus, these motoneurons demonstrate both programed and reflexive patterns of activity. Contraction of the ABEMs will facilitate the movement of water through the gills by expanding the roof of the gill chamber in a diaphragm pump-like manner. (N.S.E.R.C. Canada)

IN VITRO CONNECTIONS BETWEEN LYMNAEA AND HELIOMA NEURONS. Bulloch, A.G.M., Syed, N.I. and Lukowiak, K. Dept. of Medical Physiology, H.S.C., Univ. of Calgary, T2N 4N1

In vivo, the giant pedal dopaminergic interneuron (R.Pe.DI) of the pond snail Lymnaea stagnalis makes monosynaptic connections with its follower cells in the parietal and visceral ganglia. These follower cells of R.Pe.DI in Lymnaea are identified and their connections are well characterized. In order to test the appropriateness of these connections in vitro, the R.Pe.DI and its follower cells were cultured in Lymnaea conditioned medium. Later, the R.Pe.DI was found to make chemical connections with its follower cells. In related studies, the neuron homologous to Lymnaea R.Pe.DI was found in the pond snail Helisoma (L.Pe.DI). The follower cells of Helisoma L.Pe.DI have been located but their connections have yet to be characterized. In Lymnaea, R.Pe.DI was found to be predominantly excitatory to the pyloric ganglion (L.Pe.DI). In Helisoma, however, the route taken by the R.Pe.DI was not found to be predominantly excitatory to the pyloric ganglion (L.Pe.DI). The following studies characterize the ability of homologous cells from two different species to establish chemical connections, which appear specific.

IN VITRO RECONSTRUCTION OF THE CENTRAL PATTERN GENERATOR (CPG) UNDERLYING RESPIRATORY BEHAVIOR IN LYMNAEA, N.I. Syed, K. Lukowiak & A.G.M. Bulloch. University of Calgary, Alberta T2N 4N1, Canada

An interaction between inhibitory and excitatory rhythm generators has been shown to produce the mammalian respiratory rhythm, but little is known of the circuitry within these CPGs. The intraneuronal circuitry underlying respiratory behaviour in the pond snail Lymnaea has already been described. The interneurons IP3 and VD4 have reciprocal inhibitory connections with each other and are implicated in the opening (expiration) and closure (inspiration) movements of the pneumostome. The interneuron R.Pe.DI can initiate the respiratory cycle by post-inhibitory rebound excitation (PIR) of the IP3 neuron, which in turn excites VD4 by PIR and the cycle repeats. In order to test the sufficiency, necessity, adequacy and modulatory role of these neurons in the CPG, these identified neurons were cultured in vitro. All these cell types exhibit extensive neurite outgrowth within 18-24 hrs of plating in conditioned medium. When all three cell types were plated together in the same dish it was possible to initiate the respiratory cycle by the excitations of R.Pe.DI and the network became coupled, IP3 and VD4 interneurons bursting alternatively. This mimics the situation during in vivo active respiratory behavior. These studies therefore suggest that at least a relatively simple CPG can be reconstructed in culture and its activities can be studied and manipulated in greater detail.
416.9


The pedal ganglia of Helicoma trivolis contain a network of GABA-accumulative neurons. To date none of the Helicoma pedal neurons have been characterized. However, in the related basommatophoran pulmonate, Lymnaea stagnalis, the movement of pedal neurons in respiratory and locomotory activity has been recently demonstrated. The circuitry underlying these behaviors has been partially characterized and there provides an opportunity to investigate the role of GABA in modulating the patterned activity generated in the pedal ganglia. This was accomplished by bath application of GABA while recording intracellular activity from peddle and visceral neurons known to be involved in the respiratory and locomotory rhythms. GABA caused an inhibition of spontaneous respiratory rhythms. In addition, cycles of the respiratory rhythm stimulated by depolarization of the right pedal neuron 1 (R. Pe.DI) were abolished in the presence of GABA. GABA induced hyperpolarizations of both the right and left Fe.DI neurons. Neurons of the pedal A and D clusters of the locmotory CPG were also hyperpolarized by GABA. These preliminary data suggest that GABA has inhibitory effects on both respiratory and locomotory CPG neurons.

416.11


Fictive locomotion in the lamprey spinal cord has been entrained by imposed movement. Data on a) entrainment frequency ranges, and b) phase coupling between forced movement and ventral root bursts, were used to test a mathematical model of the CPG as a chain of coupled nonlinear oscillators. The model predicts the following, which are borne out by the data: 1) the phase lag between ventral root activity depends upon frequency; 2) the phase lag is lost at frequencies for which the phase lag goes out of a permitted range; 3) this permitted range is independent of the number of spinal cord segments in the chain; 4) the variation of phase lag with frequency depends on chain length in such a way that the entrainment frequency range decreases with increasing chain length. Additional data on entrainment frequency ranges are consistent with the model if a) interaction between segmental oscillators decreases their frequency, b) ascending coupling is stronger than descending, and c) ascending and descending coupling signs have different timing.

Supported by SERC, NINCDS, NSF, AFOSR.

416.13

CONVERGING AND DIVERGING CONNECTIONS IN NEURAL NETWORKS: HOLONOGY AND COMPUTATIONAL POTENTIAL OF THRESHOLDS AND SYNAPTIC WEIGTHS. G. J. Mitchos and R. M. Burton, Herrford Marine Science Center, Newport, OR 97366, and Department of Mathematics, Oregon State University, Corvallis, OR 97334.

Findings of gaps and variability in buccal-oral motor patterns of our experimental animal, the sea slug Nereoneurochaete, motivated us to examine the ability of neural networks to learn to transmit analog chaotic signals (Mitchos, G. J., Chen, L. H. and Burton, R. M., J. Theor. Biol. 128: 459, 1988). We show here that simple connectionist networks can also learn to perform a variety of tasks on chaotic signals using different input pathways, that they can learn several different tasks at the same time. Threshold synaptic weights were sufficient for networks to learn many tasks, but by including variable thresholds the range of tasks greatly increased, suggesting that examination of trainable neuronal thresholds in biological networks may prove fruitful, as has been examined in simple models (e.g. Mitchos, G. J. and Chen, L. H., J. Neurobiol. 12: 499, 1986). When convergence is spatially separated from divergence, as in networks containing one input unit, one output unit and only one layer of hidden units ("interneurons"), memory is distributed unevenly throughout the network. But when convergence and divergence are mixed, as between two hidden layers, memory is more holographically evenly distributed. Although training set of all synaptic weights to optimal levels, networks naturally generated many "lazy" synapses which hasted time effective learning. When presented with new inputs these synapses may be a natural product of converging/diverging biological networks, and aphrophromysom may find adaptive use in generating variable, shifting motor patterns. This work provides a more mechanistic aid to identifying, understanding, and manipulating the types of phenomena to examine in biological systems. Supported by AFOSR BV-0522.

416.10

SENSORY INPUT AND EFFERENCE COPY IN AN INSECT CENTRAL PATTERN GENERATOR. J. H. Belanger and I. Orchard, Dept. Zoology, University of Toronto, Toronto, ON, Canada, M5S 1A1.

An important problem in the study of central pattern generator (CPG) function is the interaction between central pattern generators (CPG) and other components of the nervous system. We are currently investigating this, using ovipositor/gating neurons in the locust (Locusta migratoria). The CPG network driving oviposition is driven by isolated ganglia taken from females interrupted during oviposition, but it generally ceases within 30 min. It can be restarted by electrically stimulating peripheral nerve or central ganglia. This CPG consists of sensory axons from the ovipositor, which provide input to the CNS. These nerves contain sensory axons from the ovipositor, but do not appear to contain any motor axons. The initiated rhythm outlasts the stimulation by several (often ten) minutes, and the groom can be repeated several times. This suggests sensory input may be important in either the initiation or maintenance of this centrally-generated rhythm.

We also have preliminary evidence that an efference copy of the motor output is sent to more rostral portions of the CNS. There are spikes ascending in the abdominal connectives which are time-locked with the rhythmic bursts in ovipositor motor nerves. We hypothesize that this information is used to coordinate the digging rhythm with the other phases of oviposition.

416.12

LONG-TERM PERIODICITY IN NEURAL NETWORKS WITH STRONG INHIBITION. D. P. Bashor and Q. Tang* Dept. of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina 28223

Neural network simulations lasting tens of sec extend the work of RJ MacGregor and TD McMillen, Biol. Cybern. 28: 121, 1978. 13 excitatory (E) cells and 12 inhibitory (I) cells were connected in 2 different patterns, and the response of these networks was examined as mean random E and I input varied. The mean number of E synapses, I synapses and total synapses was fixed at one of 3 levels. Synaptic strength was fixed, E at 0.2 of threshold and I at -0.7. All cells in Pattern 1 synapsed randomly with both kinds of cells. Each cell could make multiple synapses and self connections. In Pattern 2, E cells only synapsed with randomly chosen I cells, and I cells only synapsed with randomly chosen E cells.

A range of E and I input values caused both networks to produce spikes in bursts with mean cycle lengths up to about 300 msec. Mean interburst gap (GAP) was about 140 msec for both patterns and all synaptic densities. Bursts shortened but GAP did not change in both patterns with increased E input. Increased I input lengthened mean GAP. In Pattern 1, mean burst duration went from 204 to 261 msec as number of synapses went from 250 to 375, then decreased to 171 as synapse number went to 500. In Pattern 2, mean burst duration increased from 80 to 280 msec as synaptic density increased.

416.14

MODEL NEURAL NETWORKS FOR ADAPTIVE BEHAVIOR. B. D. Beer.1 H. J. Chiel.2 and L. S. Sterling.1 Depts. of 1Computer Eng. & Sci., 2Biology, and Ctr. for Automation and Intelligent Systems Research, Case Western Reserve University, Cleveland, OH 44106

We have used neuroethological and neurobiological data to design model neural networks that can generate complex, adaptive, and autonomous behavior. We have devised a simulated insect and several controllers using neuron-like elements. The pattern generator, or CPG, uses the input of sensory signal, and the controller to generate bursts shortens or lengthens the bursts, but the duration of the bursts remains fixed. The model insect can appropriately integrate different behaviors. For example, if it encounters an obstacle while following a odor gradient, it can follow around the edge of the obstacle, and then continue following the odor gradient. This model is more detailed and realistic so that it can be used to generate experimentally testable hypotheses about the neurobiology of insects and their mechanisms useful ideas for the design of artificial devices capable of adaptive, autonomous behavior.

The lamprey nervous system in vitro serves as a model for the cellular basis of vertebrate locomotion. Briefly the segmental premotor interneurons which activate motoneurons during fictive locomotion are rhythmically active and their interactions can explain the segmental burst generation (Buchanan & Grillner, 1987; Grillner et al., 1988). This network is subject to a powerful sensory input from receptor neurons (Grillner et al., 1981; 1984), which provides afferent monosynaptic EPSPs on the network. The reticulospinal initiation is exerted via monosynaptic EPSPs in all network interneurons (Ohba & Grillner, 1989). Efference copy information from the spinal level modulates the reticulospinal discharge (Dubuc & Grillner, 1989). Realistic simulations of this entire circuitry has been made by modelling each type of neuron, with relevant types of membrane channels (Na⁺, K⁺, Ca⁺, Ca²⁺), conventional EPSP and IPSP channels, and voltage dependent NMDA channels. The latter are of particular importance during slow swimming (Brodin & Grillner, 1985, 1986; Sigvardt et al., 1985; Wallen & Grillner, 1987). The cells have been and will be implemented in parallel. The network simulates a large variety of experimentally established findings.

416.16 COMPUTER SIMULATION OF OSCILLATION IN MUTUALLY INHIBITORY HEART INTERNEURONS IN LEELCH. E. De Schutter*, T.W. Simon, J.D. Angstadt and R.L Calabrese (SPON: P. Lennard). 1Dept. of Neurology, University of Antwerp, Belgium,*Dept. of Biology, Emory University, Atlanta, GA 30322.

Heart interneurons (HIN) of the medicinal leech (Hirudo medicinalis) oscillate between bursts of action potential firing and periods of IPSP-mediated hyperpolarization. This is caused by the interaction of endogeneous membrane currents with reciprocal inhibition. Some of these currents have been fully characterized (Angstadt et al., Simon et al., this meeting).

The initial model consisted of only two or more compartment neurons, each with membrane currents. The equations describing a fast and a slow Iₚ and the hyperpolarization-activated inward current Iₚ could be based upon voltage clamp recordings in HN cells. The other currents, Iₚ, Iₚ (delayed rectifier) and Iₚ were adapted from equations developed for mollusk neurons (Connor and Stevens, 1971, J. Physiol. 213: 31). Amplitude of synaptic conductance was determined by transmitter release, which was a function of presynaptic voltage.

We will improve the model by using currents measured in HN cells for Iₚ and Iₚ and better representation of transmitter release when more experimental data are available. The model is used to examine the role of Iₚ in timing of the oscillatory frequency and to ascertain if Iₚ mediated repolarization of the inhibited HN cell is sufficient to cause the switch in firing from one to the other HN cell.


Recent studies have disclosed changes in currents of cortical neurons during Pavlovian conditioning. One current is reduced by anandamide and resembles g₂K. Acetylcholine and Gprotein dependent protein kinase have been shown to reduce this current. We incorporated features of this outward current and its adaptive response to accumulated second messenger into a 6 x 6 real time network. Properties of the connections between elements (the sign, amplitude, duration, and transmission delay of the PS) were individually specified to concomitant electrophysiological observations. The simulated current activated in proportion to depolarization, inactivated with sustained depolarization, and was active over a restricted range of membrane potential. Adaptation of the current was proportional to the product of the outward current and accumulated second messenger, which was in turn a function of excitatory inputs and exponential decay. Preliminary results indicated that the network by pairing a "unconditioned" stimulus (US) with an "unconditioned" stimulus (UCS) could acquire a new, conditioned response to the CS. It was also observed that the properties of the A-current only marginally affected the passage of sustained UCS depolarizations to the output layers but substantially reduced the magnitude and duration of more transient CS depolarizations before conditioning.

416.18 SIMULATION OF 1000-NEURON INHIBITORY-FEEDBACK NETWORK REVEALS MEMORY PROPERTIES: COMPUTER MODELING INSPIRED BY NEUROSTATISM. C.D. More, S.P. Sawyer, D.J. Woodard. Dept. of Cell Biology and Anatomy, UT Southwestern Medical Center.

This study was performed to determine functional properties of a three-dimensional array of mutually-inhibitory-inhibitory structure was inspired by the extensive inhibitory feedback network of the medium spiny neurons in the neostriatum. Each neuron was represented by a linear RC circuit in a simple integration and inhibition impulses, but without long current decays which cause intrinsic oscillation. The neurons communicated over limited distances via inhibitory synaptic connections. Activation was provided by a monosynaptic excitatory input (equal in excitatory and inhibitory effect) for each neuron and an external pulsatile excitatory synaptic conductance under control of the user. The circuits were performed using a Unix-based computer, with results displayed graphically. These experiments examined the effects of increasing noise, increasing anatomical feedback range, and external excitatory input. Increasing noise led to a "binary" on/off switching phenomenon in the firing of neurons in the array, the global pattern of which evolved to a more probable on/off pattern, or "state". Increasing the feedback range led to a high number of "on" cells in the pattern. A short, powerful excitatory input to a cluster (or even a plane) of cells in the network changed the subsequent sustained pattern of activity with respect to a control pattern over the length of the experiment. This network demonstrates maintained patterns of activity produced by background synaptic activity, as represented by the noise. One hypothesis is that these activity-dependent states of the network represent a model for short-term memory which acts in parallel with long-term mechanisms, such as the putative mechanism involving long term potentiation.

Support from the Biological Humanities Foundation, DA2338, and DA3532.


We have developed a flexible neural systems simulator, written in C, running under the Unix operating system designed to facilitate the construction of neural models either with emphasis on the organization and function of real neural networks using computers which are readily available to neurobiologists (cf. Wilson and Bower, AIP Press 1988). Architectural design allows rapid, interactive specification and display of network circuitry and parameters. The built-in interpretable simulation language provides access to simpler neuronal elements. The simulation language enables generating output in the form of spike activity, EEGs, intracellular potentials and field potentials to simulate the comparison of modeled results to physiological observations. The hierarchical representation of neuronal components allows simulations ranging from single channels to detailed single cell models to large networks of simple or complex cells. The module structure allows new components to be easily added to the existing system. Current models developed under this system include mammalian olfactory bulb and cortex, inferior olive, and interneural neural structures. (work supported by NSF grant EET-870066, and the Lockheed corporation)


Single unit studies show that the extracorporeal ncones run carry an eye position signal, whereas many oculomotor neurons in the brain stem encode only an eye velocity command. The eye position signal is derived from the eye velocity signal by means of a neural integrator located in the vestibular and prepositus hypoglossal nuclei. Models of the neural integrator have been constructed which are robust and accurately reproduce its long time constants. The model neurons are described by first order linear differential equations and are connected by synapses hardened in a reciprocal (feedback) inhibitory fashion that allows the network to process a signal varying in time.

We modeled the integrator as a neural network capable of learning. Learning rules such as back-propagation are unphysiological, only useful for feedforward networks and do not generate functions of time. We used the difference between actual and desired eye position, or retinal image slip, integrated over time as the error. Synaptic weights were altered successively to reduce this error in a network containing feedforward and feedback pathways. A two neuron network, using simulated vestibulo-ocular reflex inputs, successfully converged to perform integration, and we are currently working on a multi-neuron model.
417.1

The intracranial and intermedial consequences of long term tactile restriction were investigated. The mystacial vibrissae of rats were bilaterally removed at an early age, either by plucking or cauterization of the follicless, one subcutaneous injection of pentobarbital, and behavioral assessment was carried out when rats reached adulthood. Chronic restriction of somatosensory input was found to significantly affect the somatosensory system, as well as the visual system, particularly with respect to orientational/attentional responses. The treatment has less impact on certain spatial and precise manipulatory skills which were also tested. Following behavioral assessment, dendritic branching in both the somatosensory and visual cortex was measured. Elimination of sensory input from vibrissae appears to affect dendrite branching in both the somatosensory and visual cortices.

417.3

We have previously shown (Crandall et al., Neurosci. Abstr., 14:476) that a pattern of radial glia and cortical neuronal dendrites appears in barrel cortex at the same time that the cellular pattern of barrels emerges, i.e., postnatal day 4 (P4). We wished to examine the effects of surgical removal of the row C whiskers on the development of this pattern. Serial tangential sections were immunolabeled with monoclonal antibodies specific for MAP2 (neuronal dendrites and somata) and RC2 antigen (radial glia) 6 days after whisker removal on P1. The typical spatial pattern is evident in the unaffected barrels in adjacent rows B and D. Both glia and dendrites appear to be more densely distributed toward the walls (sides and septae) than the hollows (centers) of individual barrels. This non-random distribution within barrels adjacent to the deafferented barrels is less clear for the glia than for the dendrites. In contrast, the dendrites and glia in the region of where row C barrels would normally form do not show an obvious spatial distribution. These results suggest that developing thalamocortical afferrants not only influence the development of cellular pattern in the barrel cortex but also play a role in determining dendritic patterns of immature projection neurons and radial glia.

Supported by the NIH (NS 24386 and NS 12005) and a C.A. King Trust Fellowship.

417.5
EMBRYONIC RAT NEOCORTEX TRANPLANTED HOMOTOPICALLY INTO NEWBORN NEOCORTEX DEVELOPS AREAS APPROPRIATE FEATURES. B.L. Schwarz* and D.O.M. Ogawa (SPON: R. Globus) Dep of Neurosurgery, Washington Univ Sch Med, St. Louis, MO. 63110.

Embyronic rat visual cortex transplanted to the somatosensory region of newborn rats did not develop appropriate somatosensory characteristics. Barrels are aggregations of layer 4 stellate cells and thalamic afferents unique to rodent somatosensory cortex. We tested whether the observed barrel-like features are the result of normal segregation per se by performing homotopic transplants of parietal and occipital cortex. A piece of cortex was removed from E17 donor fetuses which were exposed to 3H-thymidine (4.8 M) in utero at E15. The donor cortex was placed in a cavity aspirated in a homotopic region in a newborn host. Hosts were perfused on P12 and alternate brain sections processed for ACHE histochemistry which was found to label (neuronal somata) and Nissl staining which shows cortical cell distribution. In parietal cortex, ACHE reveals the segregation of ventrobasal thalamic afferents, whereas in visual cortex it shows the homogeneous geminculocortic formation. Aggregates of layer 4 stellate cells correspond with the disjunctive pattern of intense ACHE staining in somatosensory cortex. In visual cortex, the continuous band of layer 4 stellate cells corresponds to the homogeneous pattern of ACHE. The distribution of layer 4 stellate cells correlates with the homogenous pattern of ACHE. Transplants are delineated by autoradiography of adjacent sections. Homotopic parietal and heterotopic occipital-to-parietal transplants form barrel-like structures (B-L. Schwarz* and D.O.M. Ogawa). 30 days post-transplant, cortical neurons with intense ACHE staining equally well. However, homotopic occipital transplants do not contain barrel-like morphologies. These results suggest that transplanting cortex does not cause normal somatosensory morphologies. Rather, embryonic occipital or parietal cortex transplanted to the neonatal parietal region form barrel-like structures due to the influence of ventrobasal thalamic afferents. (Supported by NINCDS grant 5 PO1 NS17783. & The McKnight Foundation)

417.6
DISTRIBUTION OF TYPE II CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE - IMMUNOREACTIVE NEURONS IN RAT SOMATOSENSORY CORTEX. C.A. Hunt and M.R. Kennedy. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Type II calcium/calmodulin-dependent protein kinase (CaM kinase) is one of the most abundant brain protein kinases, and is particularly concentrated in neocortex, hippocampus, and substantia nigra. It is highly specific for a number of brain functions including neurotransmitter receptors, signal transduction, and ion channels. The distribution of CaM kinase- immunoreactive neurons is present throughout the brain, from brainstem to neocortex. The presence of CaM kinase in the neonatal period is not known. The distribution of CaM kinase-immunoreactive neurons in the adult cortex is heterogeneous, with the highest density in the somatosensory cortex. The distribution of CaM kinase-immunoreactive neurons in the adult cortex is heterogeneous, with the highest density in the somatosensory cortex. The distribution of CaM kinase-immunoreactive neurons in the adult cortex is heterogeneous, with the highest density in the somatosensory cortex.

417.7

Serotonergic (SHT) innervation of rat somatosensory cortex (SmI) in particular dense in lamina IV during the early postnatal period (Vogelaar et al., Brain Res. 34:2372, 1978). The purpose of this study was to determine whether neonatal depletion of SHT would influence development in SmI. Rat pups received one subcutaneous injection of p-chloroamphetamine (PCA; 100 mg/kg) on the day of birth and a second injection 24 h later. The brains of PCA-treated rats and control littermates were studied 20 d later using cytochrome oxidase histochemistry, Golgi impregnation or Nissl staining. Anterograde HRP labeling of thalamocortical axons was also accomplished in PCA-treated and control rats. Three d after PCA treatment SmI SHT is reduced to 35% of normal levels. Some recovery of SmI SHT occurs but a permanent loss of 25% remains into adulthood. Despite the loss of SHT, thalamocortical patterning in SmI appears normal in both cytochrome oxidase preparations and after anterograde HRP labeling. Likewise, barrel formation by lamina IV granule cells was normal. Preliminary analysis of apical dendrites of lamina V pyramids suggests that SHT depletion results in fewer barrels (pill) than in normal SmI. Since fewer dendritic branches could reflect delayed development intermediate time points are being studied. Support: NS 25752 and DE 07734.
DEVELOPMENT OF SI FORELUMBAR REPRESENTATION IN NORMAL AND DEAFFERENTED NEONATAL RATS AS STUDIED USING PEANUT AGGLUTININ (PNA). B.A. Calford and R. Tweedale, Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Qld. 4067, Australia.

In adult mammals a small amplification, such as that of the thumb in a flying-fox or a toe in a rat, usually produces a rapid expansion of receptive fields at primary somatosensory (SI) cortical loci which originally represented the denervated area. This expansion is also seen when a small body region is denervated for the first time. Here we report that in anesthetized flying-fox not only is the representation of the denervated region in contralateral SI altered but that the representation of the mirror image body region, in cortex ipsilateral to that denervated, also shows an expansion. In normal physiological conditions we cannot show an effect of stimulation upon ipsilateral SI in the form of direct excitation, direct inhibition, or synaptic inhibition. The expansion and contraction of the receptive fields within the representation of the body region contralateral to that denervated shows the same time course, and no direct effect in those cases where local anesthetic was used to create a temporary effect; when anesthetic was used the ipsilateral expansion also retracted although the period of expansion varied considerably. One simple aspect of the result which has consequences for the general understanding of plasticity in adult brain is that under conditions where conventional physiology could not demonstrate the influence of a pathway that pathway has been shown to have an inhibitory influence upon viable inputs and indeed is one of the influences which shape receptive fields.
DIFFERENTIAL RESPONSIVENESS OF SII FOLLOWING SI LESIONS IN INFANT AND JUVENILE MACAQUES. K. Sathian, Shao Dian-Hua* and H. Burton. Dept of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

In monkeys, the second somatosensory area (SII) depends on inputs from anterior parietal cortical areas 3a, 3b, 1, and 2 (*2) for its activation. Thus, if the hand representation of SI is removed, stimulation of the hand no longer drives neurons in SII. In contrast, neurons in SII of cats are unaffected by the removal of SI cortex. Because of this dramatic species difference, we are continuing a comparative analysis, and report here on the effects of SII lesions on the responsiveness of neurons in SII in the tree shrew. In these experiments, we first mapped, in anesthetized subjects, the forelimb representation in the hand area, and then lesioned the forelimb representation in SII and then lesioned it by aspiration. We then remapped the SI hand representation. In all 5 cases of neurons in SI with receptive fields on the forelimb, the forelimb representation remained highly responsive to cutaneous stimuli after the lesion. These results, together with those mentioned earlier for monkey, suggest that neurons in SII are independently activated by thalamic projections in most mammals and that a dependency of SII on SI may be present only in monkeys. Supported by the Medical Research Council of Canada.
418.9
SINGLE NEURON RESPONSE PROPERTIES IN SENSODORITOR CORTEX IN AWAKE CATS. J.C. Sjimp and A.L. Towm. Dept. of Physiology and Biophysics, Univ. of Wash., Sch. of Med., Seattle, WA 98195

Three quasi-planar arrays of 10 closely-spaced microelectrode tracks were run in primary somatic and pericruciform somatosensory forelimb cortices in two awake, passively restrained domestic cats. Guide chambers for the electrode carriers were affixed to the cranium while the cats were under deep barbiturate anesthesia. The modality selectivity, receptive field (RF) location and size, and mode of arousal were noted for 504 neurons isolated at 337 sites (2 or more neurons were isolated at 120 sites). The spatial distribution of these response properties failed to show any clear organization other than somatotopy. Quantitative tests of the predictions of a strict columnar and of a random distribution model showed no difference from the random model, in all three arrays. Few simultaneously-recorded neurons shared the same modality and RF; 3/23 had limited or no RF overlap and 5/50 showed different modality sensitivities. Many pairs with modality sharing showed limited or no RF overlap, and many pairs with partial or complete RF overlap did not respond to the same modality. The data failed to support a model featuring local bounded regions within which modality sensitivity and RF location and size are the same. Local clusters sharing the same response properties were not excluded.

418.10
AN ANALOG-PHYSIOLOGICAL STUDY ON THE FUNCTIONAL MATCHING BETWEEN RECEPTIVE FIELDS OF SI II NEURONES AND ASSOCIATION INPUT FROM SI IN CATS. P. Barbarese, S. Bernardini, T. Mangoni. 1st. of Human Physiology, University of Ancona, Italy.

In 6 cats HRP was iontophotically delivered (2-4 μA, 15-20 min) through a micropipette (20-30 um tip diameter) into individual somatotopic zones (Zs) of SI II which were preliminarily identified by microneurostimulation. After 72-96 h several Zs of ipsilateral SI were explored with microelectrode to map neuronal RFs. Animals were perfused and their brains cut and processed for HRP. Tracer deposits in SI II were 190-450 μm in diameter. HRP-labelled cells were in all cytoarchitectural layers of SI II. Few units were found in bilateral SI and their number ranged from about 150 to 550 (counts from alternate sections). After injections in a single digit zone of SI II, labelled cells were clustered in the same digit zones of SI, except few (less than 5%) found in 2-3 of the contiguous digits. After injections in the hand or forelimb Zs, whose RFs included the digit tips, labelled cells were in corresponding Zs of SI (palm, wrist, and arm) and few (10-15%) also in the digit Zs. RFs mapped from labelled region of SI were smaller than those at the injection site, but always included in the latter.

418.11
BILATERAL INTERACTION IN CAT SII AND THE CORPUS CALLOSUM. M. Picard* F. Lepore, M. Pito, J.-P. Guillemo. Dép. de Physiologie et d'Anatomie, Univ. de Montréal, Montréal, QC, Canada

Somatosensory area SII receives ipsilateral input mainly through the corpus callosum(CC). Previous studies of cat and monkey have indicated that the ipsilateral input can inhibit or facilitate the contralateral neural response to a tactile stimulus. This experiment was designed to systematically examine this bipartite effect by comparing the response of bilateral cells to either ipsilateral, contralateral or simultaneous stimulation. The contribution of the CC to bilateral activation and bilateral interaction was also assessed by comparing normal cats to those with sectioned or intact CC. Bilateral and unilateral mechanical stimuli, equated on all parameters for a particular cell, were applied to the receptive fields (RF) of bilateral cells. Unilateral stimulation always elicited excitatory responses. The bilateral response was often stronger than the unilateral response. A cell was considered to show interaction when the bilateral response was at least 50% higher, in terms of total number of spikes evoked by the stimuli, than the stronger unilateral response. In normal cats, about one-fourth of the units showed interactive effects. Almost all RF located on the forelimbs and most were responsive to deep pressure. In cats with sectioned CC, the interactive effects disappeared almost completely, indicating that callosal input is the main source of this interaction.

418.12

Neurons in digit 3 and digit 4 cortex of cholecystokinin-immunoreactive raccoons were tested for apparent convergence using mechanical and electrical stimulation of all digits in conjunction with condition-test conditioning. Intra-arterial injections of MK-801 caused neurons to receive strong facilitatory or weak inhibitory inputs from several off-focus digits as well as excitatory input from the on-focus digit. Strongest facilitation typically occurred with CT intervals of 1-5 msec. Off-focus excitatory responses usually had lower thresholds, lower probabilities, fewer spikes and longer latencies (1-10 msec) than on-focus responses. Neurons located in "heterogeneous" hairy skin representations had larger receptive fields (RFs) and were facilitated from more digits than neurons in glabrous skin representations. Application of the GABA antagonist picrotoxin sometimes enlarged excitatory RFs to include several off-focus digits. Unmasking or strengthening of convergent inputs may account for the Sm reaction observed following peripheral nerve lesions (Kandel and Schwartz, 1984). Supported by NSF Grant BNS-8410935.

418.13
THE EXCITATORY AMINO ACID ANTAGONIST (EABA), MK-801, ATTENUATES SOMATOSENSORY-INDUCED POTENTIALS (SEPs) OF RATS. Z.A. GALLAHER, T.M. ENEMY* and R.R. NOTISW. Wyeth-Ayerst Research, Princeton, NJ 08543-8000

The purpose of this study was to determine the effect of an excitatory amino acid antagonist, MK-801, on sensory processing within the CNS. SEPs were used as a functional measure of sensory processing and were recorded 0, 30, 60, 90, and 120 min post-drug in conscious, restrained male Sprague-Dawley rats (forelimb stimulation: 2-4 mamps, 100 μsec duration, 1.7/sec). MK-801 produced a dose-related decrease in the amplitude of the 8 msec peak close to the predrug baseline was +4% for vehicle control, -24% at 0.025, -87% at 0.05, and -93% at 0.10 mg/kg ip. The effect was seen at 30 min and generally reversed by 90 min. Reduced amplitude and increased latency were observed in later peaks. Attenuation of SEPs by low doses of MK-801 suggests that EAAAs interfere with sensory processing. Other EAAAs (kainic acid and pCP) have dissociative anesthetic and psychotomimetic properties. The MK-801 change in SEPs may reflect alteration of dissociative/psychotomimetic properties of some EAAAs.

418.14

Removal of mystacial vibrissae in adult rats leads to alterations in the representation of the vibrissae in the barrel cortex. We examined changes in the distribution of growth associated protein, GAP-43, induced by vibrissectomy in adult rats. Removal of mystacial vibrissae in adult rats led to a decrease in the number of GAP-43 immunoreactive neurons in the barrel cortex. Removal of the central (C3) vibrissae was eliminated unilaterally from adult male SD rats. 6D or 14D later, the barrel cortex was sectioned tangentially and reacted to reveal GAP-43 by immunocytochemistry. In layer IV of the cortex, the barrel regions contained larger numbers of GAP-43 immunoreactive neuronal units than the other regions. The average area of the deafferented barrel regions surrounding C3 decreased by 10.0% relative to the control side (P<0.025) while the interbarrel area showed a non-significant increase (4.6%, P<0.10). No such changes were apparent with 14D survival. These data provide evidence for structural remodeling in the adult brain, suggesting that GAP-43-containing terminals sprout into barrel centers after vbx induced deafferentation of the barrel cortex. Supp. by the VA Medical Research Svcs. and N525830.
418.15 TRANSIENT EXPRESSION OF GAP-43 IN THE DEVELOPING RAT BARRELL FIELD CORTEX. R.S. Erugumala, S. Iyengar, and L. Bezanzil, M.I.T., Cambridge, MA, 02139 and Harvard Medical School, McLean Hospital, Belmont MA 02178.

GAP-43 is a neuron-specific phosphoprotein involved in development and regeneration of axonal processes. We used a polyclonal antibody against GAP-43 to study its expression in the rat barrel field cortex during normal development and after early injury to the sensory periphery. The immunostaining pattern was compared to the organization of vibrissal barrels seen with cytochrome oxidase (CO) histochemistry and Nissl stains.

GAP-43 was first evident in the differentiating layer IV (PND3) as an array of punctate densities which correspond to the pattern of vasciration on the snout, and which are confined to vibrissae barrel hollows. The densities were most intense on PND5, at which time they were surrounded by newly emerging cell-dense barrel walls. Towards the end of the first postnatal week, the staining intensity in the barrels dramatically decreased while the size of immunopositive densities increased and was comparable to that seen with CO. By PND20, GAP-43 immunostaining had virtually disappeared from the barrels while it persisted in the septum. The staining pattern was complementary to that seen with CO, and persisted into adulthood. Following partial or total row C vasciration cauter at PND0, the distribution of GAP-43 densities representing the damaged whiskers formed a continuous band. This pattern was seen as early as PND3. Based on the pattern and time course of GAP-43 expression and on the effect of peripheral manipulations, we conclude that the GAP-43 staining reflects the distribution of ingrowing thalamic afferents. Thus, the disjunctive organization of these afferents occurs prior to cytoarchitectonic differentiation of cortical barrels.

Supported by NIH grants EY05504, EY01052, and NS25630.


N-acetylaspartylglutamate (NAAG) is implicated in chemical transmission at excitatory synapses in the neocortex. We studied the morphology and laminar distribution of neurons and terminals immunoreactive to an antiNAAG serum in the somatic sensory cortex of rats and cats. Animals were perfused with 4% carbodiimide and post-fixed for several days in 4% paraformaldehyde. 25 µm-thick sections were cut and processed for IHC.

In both rats and cats, numerous NAAG-positive neurons and dot-like structures (probably axon terminals) were present in all layers, although with significant differences: neurons were densest in layers II and V, while terminals were densest in layer I. In rats, the majority of positive neurons were non-pyramidal (NP) cells, while in cats pyramidal (P) neurons predominated. These results indicate that: 1) NAAG might have a role in excitatory transmission in the neocortex, as revealed by p cells labeling; and 2) this peptide might also have other actions, as suggested by the labeling of numerous NP (presumably GABAergic) neurons.

419.1 EFFECT OF SEQUENTIAL IMAGE PRESENTATION ON TEMPORALLY MODULATED RESPONSES OF STRIATE CORtical NEURONS. J. M. Wannag, T. J. Gwone, B. J. Richmond, and L. M. Optican, Laboratory of Neurophysiology, National Institute of Mental Health, and Laboratory of Sensorimotor Research, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892.

The responses of neurons in primary visual cortex show stimulus-dependent temporal modulation: when a stimulus is presented in isolation, the waveform of the response depends upon the spatial pattern and duration of the stimulus. However, images do not occur in isolation during normal vision. Thus, it is important to know how changes from one image to another affect the responses of visual neurons. In this study, responses to stimuli consisting of sequential images were compared to the responses to isolated images. Single units in primary visual cortex were recorded in behaving monkeys. During fixation, 2-dimensional black and white stimuli were flashed on the neuron's receptive field. Stimulus pairs that gave disparate response waveforms were chosen. Each stimulus was presented in isolation for each of 16 durations. The stimuli were also shown sequentially, with varying durations (33 – 133 msec) and varying intervals between them (0 – 133 msec). The responses to the paired stimulus presentations were compared with the predictions of a neuron model, based on the linear addition of the responses to each stimulus presented alone.

When the interval between images was 66 msec or greater, the responses were independent and obeyed superposition. For shorter intervals, many were still predicted by superposition. When superposition failed, the initial peak of the response to the second stimulus was both decreased and delayed. However, this waveform returned to that predicted by superposition before its end. This suggests that less than 66 msec is required to change the temporally encoded messages about sequentially occurring images.


Shannon's information theory provides a model-free method for quantifying multidimensional stimulus-response relationships in neurons. Unfortunately, estimation of stimulus-response probabilities from data which are continuous, noisy, or few in number leads to biased overestimates of transmitted information, T. We have developed an improved estimator, T*, which corrects for these biases.

T* can be obtained from T by subtracting a term that goes up as either the sample size goes up or the noise goes down. The improvement remaining after the stimulus-response relationship has been randomized, B, is a measure of the small sample bias. If the stimulus-response relationship is random, then further randomization will have no effect and T and B will be the same; otherwise, B will be less than T. Hence the ratio (RT) can serve as a factor sensitive to the data's signal-to-noise ratio. Thus, a combination of these two factors gives the improved estimator:

T* = T – (B/RT).

T* is accurate within 5% for sample sizes as small as 7 in simulated data sets contaminated with both Gaussian and uniform noise. For neuronal data, T* was a better estimator than T for sample sizes below 30. Thus, we propose T* as a new estimator for transmitted information in biological signals since it minimizes errors caused by 1) quantization, 2) noise, and 3) small sample sizes.
419.3 LINKING SIMPLE CELLS WITH QUADRATIC MODELS OF MOTION PERCEPTION. H. Suaiter* & C. Koch (SPON: M. Kennedy).

Behavior in cats has been studied as well as psychophysical evidence (Hassenstein & Reichardt, 1956; Van Santen & Sperling, 1985) supports the notion that short-range motion perception is mediated by a system with a quadratic type of nonlinearity. However, there is little physiological evidence that quadratric nonlinearities are found in motion selective neurons. We propose a model for motion motion selective neurons, a model which predicts the existence of quadratic motion selective neurons and the behavior of these neurons in a system with quadratic nonlinearity.

419.4 A BACKPROPAGATION TRAINED MODEL SIMULATES THE RESPONSES OF DISPARITY TUNED PRIMARY VISUAL CORTEX NEURONS. D. Zipser, Cognitive Science Dept., Univ. Calif. San Diego, La Jolla, CA 92037.

Backpropagation learning has proven useful in programming model neural networks to simulate the observed responses of actual cortical neurons. While backpropagation is a supervised learning procedure, it can be used in an unsupervised mode, called identity mapping, in which the output target is exactly the same as the input pattern. The responses of the hidden units in backpropagated trained identity mapping networks represent an optimal feature encoding of the input patterns and a robust representation of the principal components of the input correlation matrix. A model neural network was trained by backpropagation to do an identity mapping using as input a pair of retinas on which a stimulus was represented with a range of disparities. The disparity tuning of the hidden units in this model network was expressed in the output of the hidden units in response to a large range of disparities. The output of the hidden units in response to a large range of disparities is expressed in the output of the hidden units in response to a large range of disparities.


In earlier experiments (Greenberg, 1981), we noted that the geniculocortical afferent receptive fields encountered in radial electrode penetrations through area 17 were commonly dispersed as to cover elongated regions of the visual field. We now attempt to relate the axis of this elongation to the fixation point, the position of the receptive field while the position of the receptive field varied with direction of gaze, the position of the receptive field while the position of the receptive field varied with direction of gaze, the position of the receptive field while the position of the receptive field varied with direction of gaze, the position of the receptive field while the position of the receptive field varied with direction of gaze.


Receptive-fields at the cat’s 17-18 border were almost 5° further into the ipsi-hemifield than those in the LGN. While callosal inputs have been shown to drive cells at the 17-18 border, we have previously found that not all cells in visual cortex depend on the LGN. In this study we examined the contribution of direct and callosal inputs to cellular activity. After locating such cells in cortex, we found the visual extent of ipsi-callosal inputs extended to the LGN at the same elevation, and placed injection pipettes under the LGN near this site in both optic tracts. Since simultaneous injection of both 17-18 border and 17-18 border Activity, we were able to unequivocally determine the fraction of cortical activity supported by direct thalamic or callosal input. On average, direct thalamic inputs were capable of supporting 8% of normal activity, whereas callosal inputs supported 25%. Control experiments suggest that the medial interlaminar nucleus (MII) is the source of most ipsi-hemifield activity along the 17-18 border. The fact that the MII relays ipsi-hemifield activity only for the contral-eye may explain why only the contral-receptive fields extended well into the ipsi-hemifield, even though most cells in this study were binocular. (Supported by NIH grant EY08695)

419.7 RESPONSES OF NEURONS IN PRIMARY VISUAL CORTEX ARE INFLUENCED BY EYE POSITION. T. B. Meyand and J. G. Malpeli.

Department of Psychology, University of Illinois, Champaign, IL 61820.

We have investigated the possibility that eye position influences the visual responsiveness of neurons in cat area 17. With their heads fixed and direction of gaze monitored via the scleral search technique, cats were trained to maintain fixation on a small laser beam projected onto a rear-screen. When a single neuron was isolated, behavioral relevance to the animal. For 7 of 40 neurons, the tested visual evoked by stimuli moving through the receptive field varied with direction of gaze, the position of the receptive field varied with direction of gaze, the position of the receptive field varied with direction of gaze, the position of the receptive field varied with direction of gaze, the position of the receptive field varied with direction of gaze.


Strong evidence supports the concept that Area 17 and 18 of cat visual cortex perform qualitatively similar neuronal operations at different spatial-temporal scales (Movshon, Thompson and Tolhurst, J. Physiol. 1986; McLean and Palmer, ARVO ’88). Recent evidence suggests that PMLS and PLLS contain unit populations with partially non-overlapping spatiotemporal response optima (Zomborich and Blakemore, J. Neurosci. ’87). In order to demonstrate the known spatiotemporal segregation between Area 17 and 18, we recorded from at least a factor of two. Effects on response magnitude were mainly observed for horizontal gap shifts. For 6 of the 7 neurons, the larger response was obtained when gaze was directed contralaterally from the hemifield in which the eye position was fixed. This effect was most pronounced for the neuron with the largest receptive field. Eye position affected general excitability, since for some cells spontaneouse activity also varied as a function of direction of gaze. These gaze-related responses may serve to construct a head-centered frame of reference. (Supported by NIH Grants EY05818 and EY06959.)
RESPONSIVENESS OF CELLS IN AREA 18 AFTER LOCAL INACTIVATION OF AREA 17 IN CATS. I. Michaud, C. Cercone, P. Holzhüter & M. Calosetti, Université de Montréal, Département de Sciences Biologiques et Centre de Recherche en Sciences Neurologiques. Montreal, Canada H3C 3J7.

It is well known that area 17 or striate cortex projects on a cortical area as area 18 or V2. However, very little is known on the functional roles of these forward connections. We have studied the effects of a reversible inactivation of area 17 on the responsiveness of cells in the second visual area (V2). Experiments were carried out on anesthetized and paralyzed adult cats. Tungsten-in-glass microelectrodes were used to record extracellular single-spike activity in V2. An injecting-recording micro pipette filled with a stained lidocaine solution was placed in a retinotopically corresponding region in the upper layers of the striate cortex. So far, a total of 20 cells were tested. Out of these, 11 and 9 units were classified as simple-like and complex-like cells, respectively. Our results indicate that the response properties of one-third of the simple cells were modified after the blockade of area 17. In a few cases (3), responses were totally abolished. On the other hand, receptive field properties of only one-third of the simple cells were modified by the inactivation. These preliminary results suggest that the disruption of the feedback connections is more likely to affect the complex cells of the second visual area. (supp. CRSNG and FOAR to SM; MRC to CC)

DIFFERENTIAL RESPONSE OF CAT VISUAL CORTICAL NEURONES TO MONOCHROMATIC AND POLychROMATIC MOVING BACKGROUND - A NEW METHOD FOR RAPID ASSESSMENT OF COLOR SENSITIVITY. H.J. Koch* & H.R. Dünse (Spon. ENA) Strahlenklinik, University of Ulm, West-Germany, and Coleman Lab, USCF, San Francisco CA 94143.

Because of their nocturnality, cats color vision is not highly developed or does not provide a very compelling source of environmental information. However, cats clearly possess some form of color vision, the nature of this capacity is not fully understood. It has been argued that cats can discriminate color only if the stimulus subtends a fairly large visual angle (Loop & Bruce, Science 199: 1221).

We have studied the differential response of cats color neurons to monochromatic and polychromatic stimuli. It is well known that the visual background.

Our results indicate that movement sensitivity may strongly depend upon the visual background.


Neurons of the visual cortex possess fine tuned trigger features which evoke optimal responses. These properties may be modified if a second stimulus (S2) is introduced outside the classical receptive field (CRF) boundaries. This study was aimed at analyzing the responses to a moving light bar when a second conditioning stimulus was also present. Rabbits were anesthetized and prepared for single cell recordings. The first, or test stimulus (S1) was a light slit moving back and forth at the optimal orientation in the receptive field. S2 moved in phase with S1. When presented in isolation, S2 had no effect on spontaneous activity of the cell. But, when the stimuli were applied simultaneously, responses to S1 were significantly altered (modifications > to 25%). Test responses may be increased (X = 62.6 vs 28.3) or decreased (X = 68.5 vs 21.9, N = 46). Mobile conditioning stimulus had a more pronounced influence than stationary stimulus (68.85 vs 45.3) F < .005, N = 25.

As the index of directionality increased, the alteration became stronger, and the preferred direction was most strongly affected. The simple cells (N = 6) and the end stopping units (N = 3) were the most influenced while only 36% (N = 19) of complex units reacted to S2. These results indicate that movement sensitivity may strongly depend upon the visual background.

DIFFERENTIAL DETECTION OF SINGLE CELLS IN THE MACAQUE STRIATE CORTEX. D.P. Edwards* and Eudh Kaplan (SPON: J. Gordon), The Rockefeller University, New York, NY 10021.

Input to striate cortex of primates consists of two distinct streams: Magnocellular (M) and Parvocellular (P). Whether the M and P streams retain their separate identities or are combined in the striate cortex is a matter of current interest. We sought to determine for cytochrome oxidase and the electrode tracks reconstructed. Micropipette filled with a stained lidocaine solution was placed in a retinotopically corresponding region in the upper layers of the striate cortex. So far, a total of 20 cells were tested. Out of these, 11 and 9 units were classified as simple-like and complex-like cells, respectively. Our results indicate that the response properties of one-third of the simple cells were modified after the blockade of area 17. In a few cases (3), responses were totally abolished. On the other hand, receptive field properties of only one-third of the simple cells were modified by the inactivation. These preliminary results suggest that the disruption of the feedforward connections is more likely to affect the complex cells of the second visual area. (supp. CRSNG and FOAR to SM; MRC to CC).


It has been suggested that competitive inhibitory interactions between orientation-tuned cortical neurons may be used to increase their orientation selectivity. One prediction of such models is that the orientation selectivity of the initial neuronal response should be relatively low, but that selectivity should increase over time, a suggestion supported by recent data from anesthetized, paralyzed cats (Best et al, in Neural Networks from Models to Applications, I.D.S.E.T. 1989). We have further tested this hypothesis by analysing the responses of neurons in area VI of the awake primate. Stationary or drifting square-wave gratings, 7° in diameter were presented fixationally for 1 second while the animal was performing a saccadic task, and the responses of 102 neurons analyzed. Eighty-six had visual responses, typically with latencies in the range 40 to 85 ms (mean = 58.5 ms) although some had longer latencies (up to 150 ms). Approximately 75% of these responses were orientation specific. We analysed in detail the responses of 24 neurons for which at least five repetitions of all 16 test orientations were available. Mean activity during fixation was about 7 spikes/s. Within 10 to 20 ms of the start of the response, mean activity increased to about 100 spikes/s for the optimal orientation, while decreasing to only 2 spikes/s for the non-optimal one. Indeed, the largest difference between the responses to the optimal and non-optimal stimulus was seen after 10 to 20 ms. We were also able to estimate the bandwidth ± half-height which had an average value of approximately 45° (range 15° to 72°). For many cells, this bandwidth was as small as the start of the response as it was later on. It is unclear why our results differ from those of Best et al, but the difference could be due to a species difference, or an effect of anesthesia. However, our results indicate that orientation selectivity can be generated without the use of feedback loops and probably does not require competitive interactions between orientation selective units.


We have used 2-D spatial/temporal N-ary noise to visually stimulate cortical areas of the macaque monkey. Stimulus-response cross correlation functions were then computed using the methods of Lee & Schetzen (Int. J. Circ. Th. 2:237-254, 1965). The resulting (three-dimensional) first order and (six-dimensional) second order cross correlation functions have been used to visualize the details of the space-time structure of the interaction within the receptive fields of V1 cells. Examples of simple, complex, and unoriented cells will be shown using dynamic and static visual displays. Methods will be presented that employ such cross-correlation results to evaluate and parameterize nonlinear structural models of macaque visual neural networks. Supported by NIH grant EY05156 and Air Force grant AFOSR-89-0247.
419.15


Neurons in the visual cortex are arranged in columnar organization according to their orientation and ocular preferences. However, little is known about functional neural connectivity through which visual information is processed between columns except a report on layer 3-6 (To's et al. 1986). Present experiments were designed to study functional connectivity of neurons in the horizontal direction in layers 3-6 of the striate cortex of cats anesthetized with 
N2O in addition to halothane when necessary. Spontaneous discharges were recorded simultaneously from a pair of cells that were separated horizontally by less than 1 mm in the cortex and interactions between them were studied by cross-correlation analysis. Many of the correlated firings showed an existence of common inputs to cell pairs separated by less than 500 µm. In this range of horizontal separation, correlated firings were observed mostly between cells located in the same layer. Also, the correlated firings were found mostly in cell pairs with similar orientation preferences in all the layers including layer IV, which receives inputs mainly from the lateral geniculate nucleus and layer V. These results suggest that cortical cells which locate in the same layer and have similar orientation preferences tend to share the same input beyond orientation columns.

419.17

FUNCTIONAL SPECIFICITY OF INTERLAMINAR CONNECTIONS IN CAT VISUAL CORTEX. Cornelius Schwarz* and Jürgen Bölz, Max-Planck-Institut, Friedrich-Miescher-Labor, 7400 Tübingen, West Germany.

In addition to the columnar connections, individual cells project over long distances parallel to the cortical surface (Gilbert and Wiesel, J. Neurosci. 3, 1116, 1983). One source of long range connections are pyramidal cells in layer 5 which project horizontally and monosynaptically to the inferior temporal cortex (Jones, 1984). Another source of long range connections is the long receptive fields of layer 6 cells which is suggested by inactivation experiments (Bölz and Gilbert, Ibid. J. Neurosci., in press). These experiments demonstrate that local excitations within layer 6 do not result in inhibition of the leading cell.

MATCHING ORIENTATION: The majority of cell pairs with correlated firing had similar orientation preference.

RECEPTIVE FIELD POSITION: The receptive fields of the layer 5 cells were within the summation area of the layer 6 cells in almost all cell pairs with correlated firings.

RECEPTIVE FIELD TYPE: Most correlations were found with layer 5 complex cells, but only rarely with layer 5 simple cells.

419.19

MAJOR EXCITATORY PATHWAYS IN THE ANESTHETIZED, SLICED AND ANALYSIS, DERIVED FROM THE LAMINAR PATTERN OF EVOKED WHITE AND GRAY MATTER. G. Vaknin and T.J. Teyler, Biophysics, Phillips University, Marburg, W. Germany 3550; Dept. Neurophysiology Lab, INSERM 94, Bron, France 69500.

Recent anatomical information on INTEGRAL area connectivity in the visual system suggests that functional analysis of coupling between neurons and neural patches. We measured coupling between cell patches of Area 17 and 18 using several techniques: local field potentials (LFP), spike-triggered multicanal evoked activity (SMA) and spike-trig-gered multicanal evoked activity (SMA) and spike-triggered mass correlation analyses (SMA). Measurements were made only during spontaneous activity.

All measures are most likely to show coupling when receptive fields (RFs) are overlapping. Here we show that in all categories of coupling, coupling is called diffuse when the MUA exhibits a broad bandwidth occurrence. This occurs when the leading cell fires and the trigger spike is focussed when a correlation peak of about 40 ms or RFs are overlapping and orientation preferences are matched. (Fig: A17 RF correlation with A18 is shown on spike-triggered mass correlation histogram 119 µs ±50 µs.)

419.20

EVIDENCE THAT GLUTAMATE IS THE MAJOR EXCITATORY TRANSMITTER IN CALLOSAL PROJECTIONS TO RAT VISUAL CORTEX. E.L. Berry, A. Y. Niemczyk, and T.J. Tyler, Dept. of Neuro, NEUOCOM, Rootstown, OH 44272.

To study the electrophysiology and pharmacology of rat visual callosal projections our laboratory has developed a slice preparation which preserves callosal fibers afferent to visual cortical areas OC1 (area 17) and OCM (medial area 18). In the present study stimulation of white matter (700 to 2500 um medial to recording tracks) yielded field potential profiles recorded with 100 um spacing in tracks perpendicular to the cortex. Recordings were collected before, during and after perfusion with media containing 1 mM kynurenic acid (KYN)--a glutamate receptor blocker. Current source density analysis of field potential profiles showed spatial localization of current sinks which were integrated to provide measures of excitatory synaptic activity. Stimulation of callosal fibers yielded major current sinks in OCM which were usually confined to supragranular layers. Perfusion of media containing KYN resulted in a 50% reduction in current density in the area of these current sinks. Callosal stimulation resulted in large current sinks in supra- and infragranular layers in OC2. A 90% wide current sink of supragranular current sinks and a 70-100% reduction of infragranular sinks followed perfusion of media containing KYN. Washout of KYN resulted in partial to full recovery of current sinks.

These data are consistent with the hypothesis that glutamate is the major excitatory transmitter in the callosal projection to OC1 and OC2.

Norepinephrine (NE) modulates neocortical activity. NE reduces the after-hyperpolarization (AHP) responsible for accommodating single unit recordings in a novel model of the visual cortex. Voltage clamp studies have shown that NE reduces Ca++ and Na+-dependent K- conductances. NE is also capable of slowing AHP in the cat senso-rineuronal cortex (Foehring, et al., J. Neurophys. 61(2):245).

Coronial slices of rat visual cortex (areas Oc and Oc2) were prepared. Stable intracellular recordings were obtained from layer II pyramidal cells. NE, 10-100 uM, or the beta-agonist, (-)-isoproterenol (ISO, 1-100uM) were perfused, or applied by pressure ejection (conic) near the neuron. NE and ISO produced small (<3mV) depolarizations or hyperpolarizations of the membrane potential, without accompanying changes in input resistance. However, spike frequency adaptation and slow AHPs were markedly reduced by NE and ISO. 100uM Timodil (beta-antagonist) perfusions prevented these reductions.

In order to examine the effects of NE on cortical synaptic activity, following a detailed dual color fluorescence immunostaining is to c-fos, -fos proto-oncogene appears to expression may provide a cellular method for assessing the activation of cortical microcircuitry. (Supported by grants from EPA and ORNL)


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TIMING OF THE PREOVULATORY SURGE OF FSH IN GOLDEN HAMSTERS. L. L. Badura, and Psychology Dept./Neuroscience Program, Michigan State University, East Lansing, MI 48824.


420.11 SUPRACHIASMATIC NUCLEUS (SCN) GLUCOSE UTILIZATION IN VITRO: CIRCADIAN RHYTHM AND EFFECTS OF TETRODOTOXIN (TTX).
In this study we demonstrate that the circadian rhythm of SCN glucose utilization previously demonstrated in vivo (Schwartz et al., J. Comp. Neurol. 1980) can be observed in hypothalamic slices containing SCN.

The effects of TTX on this rhythm are also described.

Hypothalamic brain slices were isolated from 175 gm Sprague-Dawley rats maintained on a 12:12 LD schedule for at least 3 wk. The slices were kept off the day of sacrifice and rats were decapitated in dim red light. Brain slices were pre-incubated at 37°C for 10 min, incubated with [3-14C]2-deoxyglucose (20 uCi/ml) for 30 min, and then washed for six times in the absence of TTX.

The results show that SCN SGU is lowest at CT17 (t-test on CT10), gradually rises from CT00 to a maximum at CT09 and gradually declines until CT15. Values of SGU from CT17 to CT06 are similar to those found by Lassen et al. but those from CT09 to CT15 are considerably higher. In the presence of TTX, SGU is reduced to about 60% of the values found in the absence of TTX for time points between CT17 and CT06. How does TTX affect the SCN and which regions of the brain are affected?

The circadian rhythm of SCN glucose utilization persists in hypothalamic brain slices although significant differences are observed between CT09 and CT15. In the presence of TTX, SCN SGU increases above baseline only between CT06 and CT09. These results suggest that slice isolation eliminates an inhibitory influence active in vivo during late subjective day.

420.12 IN VITRO OSCILLATION OF cAMP IN THE SUPRACHIASMATIC NUCLEI.
R.A. Prosser and M.U. Gillette. Neurological and Behavioral Biology Program, and Dept. of Physiology and Biophysics, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

The suprachiasmatic nuclei (SCN) contain an endogenous circadian clock that survives in vitro, where it continues to oscillate with a period that reflects the critical phase of the circadian cycle. Thus, it is possible to investigate the possible involvement of cAMP in the biochemistry underlying the SCN clock. We have shown previously that daily injections of cAMP increase endogenous cAMP levels in vitro phase-shift the SCN clock by up to 5 hr (J. Neurosci. 9:1073, 1989).

For further elucidation of the role of cAMP levels in the SCN, we measured cAMP in rat SCN tissue in vitro under different conditions.

Hypothalamic brain slices containing the SCN were prepared during lights-on from adult Long-Evans rats housed in a 12:12 LD cycle for 7 days. Animals were killed by decapitation, and the SCN was removed and homogenized in ice-cold 50 mM Tris buffer (pH = 7.4). Supernatants were prepared by centrifugation at 10,000 x g for 10 min at 4°C.

cAMP levels were measured by RIA. The sensitivity of the assay was 7 PM, and the intra-assay coefficient of variation was 7.3%. The assay was linear up to at least 1 nM and showed no significant variation between day and night.

cAMP levels were determined in the intact SCN and in SCN slices treated with TTX at various times of the day and night. TTX at 20 uM inhibited cAMP synthesis in intact SCN tissue, but not in slices treated with TTX at CT00 or CT06.

In conclusion, the results suggest that cAMP may be an integral component of the circadian clock mechanism in the SCN.

IN TERHESMERIC RELATIONS


Transporting a limb to a visual target involves mostly the proximal musculature which receives input from cortex in both hemispheres. The relative influence of the independent movements of the fingers and index fingers, however, depend almost entirely on projection systems from the contralateral hemisphere. Thus, early work by Brinkman and Kuyper (1973) demonstrated that the accuracy required in the contralateral hand is only possible if the hand ipsilateral to the visual scan is trained.

In this study, we investigated the contralateral hand in normal individuals with callosal agenesis or with callosotomy. Subjects were divided into two groups: 1) reaching toward and placing their index fingers on a vertically oriented disk. 2) reaching toward the disk and retrieving a knob inserted in the slot. For some blocks of trials, the disk was located in the visual field ipsilateral to the hand used being used, while in others it was located at the contralateral field. Subjects were required to maintain central fixation while reaching. Age and IQ matched controls performed the tasks with little difficulty using either hand in either visual field. The callosal and callosotomy subjects, however, were significantly more imprecise and displayed more shaping errors than their controls under all conditions. This was especially marked when the left hand was used and/or when the left visual field was the source of stimulus presentation. The results are discussed in terms of left hemisphere superiority for motor control.

421.2 DISCRIMINATION AND RECOGNITION OF COMPLEX TONAL SPECTRA BY THE CEREBRAL HEMISPHERES: DIFFERENTIAL LATERALIZATION OF ACOUSTIC-DISCRIMINATIVE AND SEMANTIC-ASSOCIATIVE FUNCTIONS IN AUDITORY PATTERN PERCEPTION. M.J. Tramo* and M.S. Gazzaniga. Program in Cognitive Neuroscience, Dartmouth Medical School, Hanover, N.H. 03756.

The discrimination and recognition of complex tonal spectra by the left and right hemispheres of two callosal agenesis patients with bilateral language competence were tested using sound-picture and sound-word matching tasks. The patients had bilateral callosal agenesis and significant hemispheric differences in language ability.

The results suggest that MEL may play a role in regulating the circadian clock mechanism in the SCN.
421.4 HEMISPHERIC ASYMMETRIES IN MEMORY FOR REAL AND SURREAL ART. Dahlia W. Zaidel and Asa Kasher*. Dept of Psychology, UCLA, Los Angeles, CA 90024-1563.

Currently there is remarkable paucity of systematic data on the cognitive and brain mechanisms underlying the reception and production of paintings. The work that has been published to date deals generally with human subjects work in pre- and post- brain damage in the same artist.

Here, the hemispheric status of the way in which two art styles treat reality was investigated with a hemifield and tachistoscopic technique. A series of 74 "surrealistic" vs "realistic" paintings was first inspected briefly by normal subjects and then memory was probed by placing the original plus decoys in the left (LVF) or right (RVF) visual half-fields. A recognition paradigm was used and a binary choice was made in response. Results showed RVF superiority for the surrealistic paintings. There was no hemi-field difference for the realistic paintings. In two additional experiments, 16 of the same paintings were used as targets in conjunction with metaphoric vs literal titles constructed for them. In one experiment, memory for the paintings alone was probed while in the other, memory for the titles alone was probed. A RVF superiority emerged for the metaphoric titles, especially if paired with surrealistic paintings. Taken together, the results suggest a left hemisphere advantage in processing meaningful, yet incongruous arrays, both pictorial and linguistic.


In spite of the association of left-handedness with neurological deficits, there is little empirical evidence for performance differences between left- and right-handers. We described the distribution of laterality in adult Brazilians and looked for its relation with performance in learning a foreign language. The distribution of handedness of 1,017 Portuguese-speaking Brazilian students undertaking of English or German courses was evaluated. We used the "abridged form of the Edinburgh inventory. In addition, we evaluated handwriting posture, instruction level, and foot preference were also obtained. Performance in learning a foreign language was evaluated by a standardized grade attributed to each student in three exams. Results revealed that the distribution of the laterality scores is J-shaped. Inverted handwriting posture was found to be a more frequent among right-handers (40%) than among right-handers (54%), The percent of dextral females (92%) was found to be greater than that of males (6%). An analysis of variance for handedness, sex, instruction level, and age showed a significant effect of handedness on the standardized performance. Strong right-handers have a better performance than weak right-handers. These results suggest that not only left-handers but also right-handers represent a heterogeneous group.

421.6 STUTTERING AND VOLUNTARY DIFFERENTIAL HEMISPHERIC ACTIVATION STUDIED WITH COMPUTERIZED EEG AND SPECT. P. S. Gott, C. M. DeGiorio,* B. C. Chen,* and E. Hughes. Dept. of Neurology, University of California Sch. of Med., Los Angeles, CA 90033.

We studied an extraordinary patient who reported he could voluntarily switch between two distinct conscious states associated with a dichotomy in fluency. f. uncontrolled stuttering, II. nonstuttering. Computerized EEG, regional cerebral blood flow (SPECT) and task performance were evaluated in each state. Findings included a state-dependent shift in left right hemispheric participation with superior results in the non-stuttering state. EEG alpha ratio was evaluated, and significantly different between states. Likewise, cerebral blood flow demonstrated hemispheric shifts in each state. Performance on verbal and spatial tasks suggested unusual lateralization.

Results support the probability that "at will" differential activation of each hemisphere was associated with each state and with concomitant stuttering and nonstuttering. Voluntary, or non-voluntary, changes in lateralized hemispheric control may be a salient feature of stuttering and of potential therapeutic significance. Partially supported by Neuroscience Corporation.


Studies in Macaques have demonstrated asymmetries in collateral processing in deaf compared with verbal visual cortex. This study examined whether visual evoked potential cortical activity would peak latency from the ipsilateral site. The distribution of latencies of 1,017 Portuguese-speaking Brazilian students undertaking of English or German courses was evaluated. We used the "abridged form of the Edinburgh inventory. Information about handwriting posture, instruction level, and foot preference were also obtained. Performance in learning a foreign language was evaluated by a standardized grade attributed to each student in three exams. Results revealed that the distribution of the laterality scores is J-shaped. Inverted handwriting posture was found to be a more frequent among right-handers (40%) than among right-handers (54%). The percent of dextral females (92%) was found to be greater than that of males (6%). An analysis of variance for handedness, sex, instruction level, and age showed a significant effect of handedness on the standardized performance. Strong right-handers have a better performance than weak right-handers. These results suggest that not only left-handers but also right-handers represent a heterogeneous group.

421.8 EFFECTS OF LATERALIZED STIMULI ON CEREBRAL AROUSAL. K. E. Lubs, J. Levy*, and E. Jepsen* (SPONSOR: P. Teuting). Department of Behavioral Sciences, University of Chicago, Chicago, IL 60637.

There is evidence that the right hemisphere plays a dominant role in the allocation of arousal to the cerebral hemispheres. Also, it has been shown that performance is better following LVF (left visual field) than RVF (right visual field) stimuli. The purposes of this study were (1) to compare the processing of verbal stimuli presented to only one and to both hemispheres, and (2) to assess the effects of uni- and bihemispheric stimulation on subsequent performance.

A tachistoscopic syllable identification task was given to right-handed men and women (n = 64). Half viewed nonsense syllables in the LVF, RVF, and CVF (center) conditions. The purposes of this study were: (1) to compare the processing of verbal stimuli presented to only one and to both hemispheres, and (2) to assess the effects of uni- and bihemispheric stimulation on subsequent performance.

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The results suggest that the visual field of stimulus presentation has an effect on the pattern of arousal of the cerebral hemispheres.

We have previously reported significant left-right volumetric asymmetries in human neonates and neonates (XT) visual area of the human brain (Soc. Neurosci, 1988; de Lacoste et al). The aim of this study was to determine if XT asymmetries in the neonatal and juvenile human brain are more pronounced in parietal neocortex (PERI) and parietal (PERI) cortex. Neonatal and juvenile (n=3) occipital lobes were sectioned bilaterally in complete series at 50µm in the coronal plane with a large-stage freezing microtome. Adjacent sectioned sections at 5mm intervals were processed using a basic Nissl and the Galván silver staining method for stain and myeloarchitectural differentiation of ST, PARA and PERN areas. Nissl-stained sections were then video-digitized and the CARP software (Biographies, Inc.) was utilized for semiautomated 1 delineation of the external and internal boundaries of cortical gray and 2) computerization of sectional areas. Regional volumes for ST, PARA and PERN cortices and left-right indices of asymmetry were calculated using previously described algorithms (de Lacoste et al, 1988).

Support for HD21711 to MCL and The Biological Humanities Foundation.


To determine whether functional asymmetries related to attentional phenomena could be shown in normal rats, forty rats were given an array of tests in order to determine the presence of consistent side biases in sensorimotor responsivity. Rats were randomly assigned to two groups: one group (n=20) received light presentations on the preferred side, the other on the neglected side. Rats receiving the light on their preferred side displayed evidence of having acquired a conditioned response to the light. Rats having received light presentations on the neglected side failed to display signs of effective conditioning. This suggests that, in a proportion of normal rats, some processes related to stimulus-reward conditioning are subject to lateralization. These effects could be useful for the study of asymmetry and its relation to psychopathology.


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Support for HD21711 to MCL and The Biological Humanities Foundation.


Exposure to 48h of post-surgical light deprivation can prevent the demonstration of neglect seen following unilateral destruction of medial agranular (Med AGm) (Crowne et al., 1983). The present study examined whether this environmental manipulation would be effective in producing recovery of function in subjects already demonstrating severe neglect.

Rat subjects (n=35) received lesion control each of six behavioral tests. Laterality ratios were derived for each task. Principle factors extraction with varimax rotation was performed on the six laterality scores. L-AGm Ss showed an ipsilesional turning preference, whereas R-AGm Ss showed no preference. These results support and extend earlier findings on laterality and recovery of function following Med AGm lesions. Supported by grant NS 24673 from NINDS to J.V.C.


The role of testosterone in determinations of behavioral asymmetry was examined in 85 perinatally gonadectomized and 91 sham male Sprague-Dawley rats. Adult animals completed four trials on each of six behavioral tests. Laterality ratios were derived for each task. Principle factors extraction with varimax rotation was performed on the six laterality scores separately for medians, and sham males. Two factors were extracted for each group. For gonadectomized rats these were interpreted as Rotational Travel and Head Rotation, while for sham animals they were interpreted as Rotational Travel and Tail Posture. The first factors were virtually identical (r = .93), while the second were unique (r = .06). Oblique rotational factors were unrelated. The divergent structure factors support testosterone involvement in head rotation and tail posture patterns, but fail to support a link between testosterone and rotational travel. Morphometric analyses of hemispheric differences in neocortical volume are in progress since behavioral asymmetry may reflect hormonallymediated hemispheric differences.


Circling in response to apomorphine (APO) was studied in rats with left or right nigrostriatal lesions (6-OHDA), who had been evaluated for directional bias prior to lesioning. Two weeks post-lesion, 21 male Wistar rats were injected with APO (1 mg/kg), and the direction of circling in an automated rotometer. Rats performing more than 30 net contraversive full turns during the 15 min. test (19 of 21 rats) were compared statistically. Animals with left-sided lesions (n=9) were found to perform significantly more net contraversive turns (mean=120.6 circles) than those with right-sided lesions (n=10, mean=91.7±0.05, one-tailed t-test). Re-examination of earlier data of APO-induced circling in the rotometer, confirmed this side of effect (p<0.05, one-tailed t-test).

Pre-examination of directional preference, based on the predominant direction of swimming in a 2-diameter pool, were found to be unrelated to post-lesion circling. The data are consistent with left striatal dominance in the population of rats as an asymmetry which may override individual directional preferences. [Supported by NSERC. RS is a Research Assoc. of OHIF].

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

Nonpyramidal neurons are known to possess the inhibitory neurotransmitter GABA and determination of whether such neurons project callosally will facilitate the development of functional schemes for interhemispheric communication. In male Sprague-Dawley rats, horseradish peroxidase (HRP) injections were made into the callosal terminal zone located near the border of areas 17 and 18. Following a 36 hour survival period, tissue sections were reacted with either diaminobenzidine (DAB), intensified with nickel ammonium sulfide and cobalt chloride, or were reacted with tetramethylbenzidine (TMB) stabilized with ammonium molybdate. Tissue sections were prepared according to standard electron microscopy procedures. Control animals were saline injected and processed in a similar fashion. More than 600 neurons possessing a Golgi-like HRP filling were examined with the light microscope to identify potential nonpyramidal neurons. Following the serial thin sectioning of four such neurons, one was confirmed by electron microscopy to be a smooth multipolar neuron. To investigate large samples of callosally projecting neurons, several series of 1 um methylene blue stained plastic sections, containing neurons displaying punctate HRP labeling, were cut from parts of areas 17 and 18 (layer II/III) and camera lucida drawings were made. The number of black HRP granules within nuclear containing profiles of pyramidal and nonpyramidal neurons was determined. Neurons in control sections did not display any granules similar to those seen in neurons with punctate HRP label. A series of adjacent thin sections were used to support light microscopic findings. Preliminary data indicate that both pyramidal and nonpyramidal neurons transport HRP in similar amounts, although nonpyramidal neurons tend to label somewhat less robustly than pyramidal neurons which could explain why nonpyramidal neurons rarely demonstrate a Golgi-like filling. (NIH-EY-06404)


Contralateral hemissectomy follows unilateral removal of all visual areas in occipital-parietal-temporal cortex. Orienting responses to stimuli in the "blind" visual field are restored by section of the tectal commissure. (Sprague '66; Sherman '74; Wallace et al. '87, '89). Midbrain commissurotomies also enhance cognitive functions. Split-brain cats show marked deficits in interhemispheric transfer of shape discriminations when measured from intact hemisphere to side with lesion in suprasylvian gyril (Berlucchi et al. '79). The known role of the superior colliculus in selective attention directed by inhibitory fibers in the commissure, suggest that restoration of transfer after collicular commissurotomies is due to enhancement of attention required to make difficult discriminations. Not yet known is whether this function, like orienting, is related to the nigrotemporal projection, part of which passes through the tectal commissure. (Supported by NIH-ET02654 and EO4896).


Previous observations suggested that the splenium of the corpus callosum is divided into two bundles by the antrum of the lateral ventricle. The larger bundle, derived from the dorsal splenium, corresponds to the classical frontal splenial major, which passes dorsomedial and dorsolateral to the atrium and occipital horn of the lateral ventricle. By contrast, the smaller bundle, derived from the ventral splenium, corresponds to the inferior forceps, which passes ventromedial to the antrum of the lateral ventricle.

To determine the sources of these two splenial components, we examined by autoradiography 33 macaques (Macaca fascicularis) with small cortical injections of [125I]-WGA-HRP. By comparing the labelled callosal projections, we were able to establish whether the source of the fiber bundle was homotopic or heterotopic. We found that fibers from the frontal cortex project predominantly to the dorsal splenium whereas fibers from the parietal and occipital cortices project to the ventral splenium. In the occipital cortex, the superior and middle temporal gyri appear to project to the ventral splenium. The inferior temporal cortex projects to the dorsal splenium. These data suggest that the ventral splenium is connected with sensory and motor regions whereas the dorsal splenium is connected with association cortex.


Alteration in the control of oropharyngeal musculature has been indirectly observed in mice after chronic neuroleptic administration by recording their licking pattern. The development of aberrant patterns parallels the time course of tardive dyskinesia: first upon withdrawal and later occurring spontaneously. This behavioral change could however, be indicative of dystonia. The two side-effects of neuroleptic administration respond differentially to increased drug concentration. Tardive dyskinesia is ameliorated and dystonia is exacerbated.

Mice chronically treated with haloperidol (1mg/kg IP) and exhibiting the altered licking pattern were challenged with a higher concentration of the same drug (3mg/kg IP). When the effects on licking pattern were compared with those of appropriate saline control groups, the number of licks per bout increased to saline levels. The higher concentration of haloperidol ameliorated the altered licking pattern.

HALOPERIDOL (HAL) SLOWED RATS' RESPONDING BY LENGTHENING THE TIME REQUIRED TO SWITCH FROM ONE MOTOR RESPONSE TO ANOTHER: A PARKINSON-LIKE PHENOCOPY OF THE PARKINSONS' DISEASE. S. Fowler, H.A. Kirkpatrick*, P.D. Skjoldager*, and R.M. Liao*. Dept's. of Psychology and Pharmacology, University of Mississippi University, MS 38677.

Classical dopamine-receptor-blocking neuroleptics decrease average rate of operant responding. Results show that by producing a total cessation of responding before a session ends and by slowing the response time intervals between the successive behaviors occurring within a typical cycle of the operant/awake/sleep cycle. Results showed that two intervals (latency from muzzle entry into the reward well to initial tongue protrusion and latency from (muzzle exit to the next forepaw response) were proportionately lengthened more than others. Hal appears to disrupt response selection and initiation processes in rats. These results provide additional evidence for homology between neuroleptics' effects in man and rat. (Supported by MH 43429)
242.2 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF PERTUSSIS TOXIN ON DOPAMINE RECEPTORS IN VIVO. C. Marin, S.A. Parsons, and T.M. Chase, ETB, NINDS, NIH, Bethesda, MD 20892.

Dopamine receptors have been classified in two subtypes: the D-1, positively coupled to adenylyl cyclase by the stimulatory Gi protein and the D-2, either not coupled or negatively coupled to the Gi protein. Behavioral experiments suggest important functional interactions between D-1 and D-2 receptors, with the D-1 system normally acting to inhibit motor activity by stimulation of D-2 receptors. In this study D-1/D-2 receptor interactions were evaluated in rats by intrastriatal administration of pertussis toxin (PT), which inactivates Gi protein linked to D-2 receptors. After unilateral injection of 1.5 μg, the animals developed a marked postural asymmetry towards the injected side. The selective D-2 agonist quinpirole (1 mg/kg, ip) produced significant ipsilateral rotation, while the selective D-1 agonist SKF 38393 (10 mg/kg, ip) had no such effect. Combined treatment with both drugs potentiates the effect of quinpirole alone. Rotation induced by quinpirole was blocked completely by the selective D-1 antagonist SCH 23390 (0.25 mg/kg, ip) and partially by the selective D-2 antagonist raclopride (1 mg/kg, ip). Treatments which resulted in rotation, quinpirole alone and with SKF 38393, also resulted in asymmetry in striatal CAMP levels. These findings provide support for a relation between D-1/D-2 behavioral interaction and alterations in CAMP levels and indicate an important role for G proteins in dopamine receptor interactions.


Yawning is a behavior modulated by Dopamine (DA) D1 and D2 preysynaptic autoreceptors. This behavior may be relevant to certain disease states. It was the aim of this study to examine these DA receptors in male Sprague Dawley rats (200 grams) treated chronically with a neuroleptic. Animals had bilateral striatal cannula implanted for injection of either the D1 or D2 agonist SKF83983 or the D2 agonist SCH23390 or haloperidol. Fluoxetine (5 mg/kg; i.p.) was injected for a 2 week period. Animals were rated for yawning and stereotypic behaviors in response to apomorphine (0.05 to 0.2 mg/kg; s.c.) three days later. Yawning behavior was inhibited by 90% after chronic neuroleptic treatment. Moreover, the D1 agonist facilitation of yawning was abolished by chronic neuroleptic treatment as was the yawning induced by the intrastriatal administration of LY171555. Yawning inhibition observed after D1 and D2 antagonist treatment was still present after chronic striatal DA sensitization. These results suggest that D1 receptor involvement as well as autoreceptor sensitization play an important role in the abolition of yawning and in certain disease states.


The present studies have compared the effects of variety of potent and selective D3-dopaminergic receptor agonists from different structural groups including an apomorphine agonist [R-N-propylpiperazine (Perfan)]], a hydroxy-naphthylbenzamine (L-745,870) and a stereoid agonist (quinpirole, quinelorane, LY173452 and LY379171) on male rat copulatory performance. The animals chosen for these studies were 10-12 month old, male Sprague-Dawley rats, which exhibited constant ejaculatory latencies (time interval between intromission and ejaculation, EL) in repeated tests. Subcutaneous injections of agonists containing various concentrations of these dopaminergic agonists were made 30 minutes prior to behavioral testing with a sexually receptive, ovariectomized female. Although all of the agonists were capable of producing significant reductions in EL, there was a 100 fold variance in the minimum effective dose. Phenylpiridine (2.5 μg/kg) elicited changes in EL in chinchilla where LY173452 required 250 μg/kg and quinelorane and LY379171 required 25 mg/kg. The overall minimal effective dose in vivo method for comparing pharmacologic activities of D3-dopaminergic agonists, which may provide a measure of preclinical efficacy of agents used to treat sexual disorders.
422.9 ANGIOTENSIN CONVERTING ENZYME INHIBITION AND APOMORPHINE-INDUCED STEREOTYPED BEHAVIOR. A. Sudilovsky, B. Turnbull, L. B. Miller*, Squibb Institute for Medical Research, Princeton, NJ 08540; Boston University, Boston, MA 02115.

Although the angiotensin converting enzyme (ACE) inhibitor captopril does not competitively bind to the dopamine receptor it attenuates apomorphine-induced stereotypy when given one hour prior to apomorphine (Sudilovsky et al. Soc. Neurosci. Abst., 91:1, 1983). The present experiments investigated 1) the role of D1 and D2 receptors in amphetamine-induced oral stereotypy and 2) the response to cholinergic stimulation of the ventrolateral striatum (VLS). One group of rats received a VLS injection of physostigmine (2 mg/kg) one hour before i.p. administration of apomorphine (2 mg/kg). A second group received a dose response relationship of the latter. In the presence of the antipsychotic dopamine receptor blocker fluphenazine at 1 mg/kg (p=0.05), which inhibits the inhibitory effect of the latter was potentiated by its combined administration with SQ 29,852. In addition, pretreatment with epicapt®pril, the virtually inactive diastereoisomer of captopril, did not significantly alter these effects. These data suggest an antagonist interaction between the ACE inhibitors and the dopaminergic system underlying stereotypy and support an involvement of ACE inhibition.

422.11 A NEUROPHARMACOLOGICAL ANALYSIS OF ORAL BEHAVIOR INDUCED BY DOPAMINERGIC OR CHOLINERGIC STIMULATION OF THE VENTROLATERAL STRIATUM. J.M. Pelfs*, C.G. Lang*, and A.E. Kelley (Spon: H. Muhle), Dept. of Psychology, Harvard University, Cambridge, MA 02138.

Amphetamine microinjection into the ventrolateral region of striatum results selectively in intense oral stereotypy. The present experiments investigated 1) the role of D1 and D2 receptors in amphetamine-induced oral stereotypy and 2) the response to cholinergic stimulation of the ventrolateral striatum (VLS). One group of rats received a VLS injection of amphetamine (20 ug) in combination with systemic pretreatment of the D1 antagonist SCH 23390, and a second group received VLS amphetamine and the D2 antagonist raclopride. Both antagonists effectively blocked oral stereotypy. In a second experiment, VLS injection of the D1 agonist SKF 38393 had no effect on behavior upon acute observation, although intense biting emerged 4 hours following injection. Quinpirole did not induce the full-blown oral syndrome, but did increase licking, head-down sniffing and biting of wood chips. Histological analysis of brain sections revealed that SKF 38393 caused extensive damage to the area surrounding the cannula tip. This neurotoxic effect may have been responsible for the delayed onset of biting. In other experiments, a combination of physostigmine and acetylcholine (PS/Ach) (0.5, 2.5, 5.0 ug) was infused into the VLS and behavior was recorded. PS/Ach induced non-directed mouth movements which were blocked by atropine.

422.12 EFFECTS OF DOPAMINE ANTAGONISTS ON ACQUISITION AND EXPRESSION OF THE AMPHETAMINE CONDITIONED PLACE PREFERENCE. Noboru Hiroi and Norman M. White (Spon: K. T. J. Pihl), Department of Psychology, McGill University, 1250 Dr. Penfield Ave, Montreal, Quebec, Canada, H3A 1B1.

Based on our previous demonstration that dopamine receptor activation is required for expression of the amphetamine conditioned place preference (CPP), we investigated the effect of selective dopamine receptor blockers on both acquisition and expression of the CPP. Using a 3-compartment CPP apparatus, rats were trained with 2 amphetamine (3mg/kg) pairings in one compartment and 2 saline pairings in another compartment in a counterbalanced manner; they were tested for CPP expression on the following day. Antagonists were given 1P before training trials (acquisition) or before testing (expression). A mixed antagonist (alpha-flupenthixol, 0.2-1.0mg/Kg), a D1 antagonist (SCH23390, 0.02-0.1mg/Kg) and a D2 antagonist (salipride, 10.0-120.0mg/Kg) all dose-dependently blocked both acquisition and expression. Higher doses of all drugs were required to block expression than acquisition; the difference was smallest for the D1 antagonist and largest for the D2 antagonist. These findings suggest that activation of dopamine receptors is required for both acquisition and expression of the amphetamine CPP. The fact that higher doses are required to block expression than acquisition suggests that different mechanisms may mediate these two processes.

422.13 EFFECTS OF SELECTED DOPAMINE D1 AND D2 DRUGS ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS BEFORE AND DURING CHRONIC SCH 39166 ADMINISTRATION. V.L. Coffin* and M.B. Letzner* (Spon: K. R. Campbell, Sheering-Plough Corp, 20 Orange St., Bloomfield, NJ 07003).

While there are a few studies on the effects of chronic D2 antagonists on schedule-controlled behavior there are none on dopamine D1 antagonists. To study this, the behavioral effects of the D1 antagonist SKF 38393 and its inactive enantiomer (SCH 39165), SKF 38393 (D1 agonist) and raclopride (D2 antagonist) were studied. Dose-effect curves for each drug were determined by administering cumulative doses sc in a single session. Acutely, these drugs produced dose-related decreases in responding under a fixed ratio (FR 30) schedule of food presentation. There was a hundred-fold selectivity between the active and inactive isomers. After these studies, daily treatment with SCH 39166 (0.1 mg/kg/day sc) was initiated. After 40 days of chronic treatment with SCH 39166, the dose-effect curve was reetermined with no rightward shift in the curve; therefore, no tolerance developed. During chronic SCH 39166, the dose-effect curve for raclopride was not altered; however, chronic SCH 39166 treatment slightly modified the effects of SKF 38393 as shown by a downward shift in the dose-effect curve. In summary, SCH 39166 itself had no effect, but the SKF 38393-induced decrease in responding due to the D1 agonist was modified by chronic SCH 39166 treatment, possibly because of the abrupt cut-off of the schedule to which tolerance did not develop over forty days during chronic administration.

422.14 DA D2 AGONIST-INDUCED DEPRESSION IS DUE TO DEPRIVATION OF DA AT D1 RECEPTORS. D.M. Jackson*, S.B. Ross* and I.G. Larson*, Astra Research Centre, Södertälje 151 85, SWEDEN.

The D2 agonists quipride (Q), pergolide (P), B-HT920 (BH) and (-)-3-PPP (PPP) produced depression (reduced sniffing, rearing and grooming) in mice using a subjective scaling system. Q-, P- and BH-induced immobility were reversed by the D1 agonist SKF38393 (D) and CY208 243 (CY). Q-induced immobilization was only reversed by the active enantiomer of S. PPP-induced immobility was reversed by CY and S, but this was due to an increase in grooming in mice given the D1 agonist, whether or not PPP was also given. There was no reversal of the depression of rearing or sniffing. While CY and S antagonized BH-induced immobility, the reverse was evidenced by an increase in grooming in mice given the D2 agonist. The depressant effects of the D2 agonists were blocked by the D1 antagonist SCH23390. High doses of SCH23390 and raclopride increased immobility. All the D2 agonists tested, in combination with SCH23390, were blocked by SC 39166. The D1 agonist (SCH23390, 0.02-0.1mg/Kg) and a D2 antagonist (salipride, 10.0-120.0mg/Kg) all dose-dependently blocked both acquisition and expression. Higher doses of all drugs were required to block expression than acquisition; the difference was smallest for the D1 antagonist and largest for the D2 antagonist. These findings suggest that activation of dopamine receptors is required for both acquisition and expression of the amphetamine CPP. The fact that higher doses are required to block expression than acquisition suggests that different mechanisms may mediate these two processes.

422.15 THE ANTAGONIST INTERACTION BETWEEN THE D1 AND D2 DOPAMINE RECEPTORS INDUCED BY AMPHETAMINE. L. Wiersma and J. Dingledine (Spon: P. T. Anden), Department of Pharmacology, University of Sydney, Australia.

We have reported that systemic administration of the D1 dopamine antagonist, SCH-23390, blocks the development of sensitization to the behavioral activating effects of amphetamine (Brain Research, in press). We now report that preexposure of rats to i.p. injections of 1.0 mg/kg d-amphetamine sulfate in the presence of microinjections of SCH-23390 (0.5 or 1.0 µg/side) into the VTA, and to a lesser extent into the SNR, attenuates the acute locomotor effects of amphetamine and blocks the development of sensitization seen in a test when only amphetamine is administered, both in a dose-dependent manner. Groups of animals were pretreated with either vehicle (VEH) or SCH-23390 (SCH) into the brain followed by amphetamine (AM) or saline (SAL) i.p. for four occasions, once every other day, and placed into activity boxes for two hours. On the tests for sensitization given two days later, all animals were treated with 0.5 mg/kg amphetamine only. These findings suggest that dopamine released from somatodendritic regions can act locally to bring about changes that may underlie the development of sensitization to amphetamine, and that SCH-23390 acts at D1 receptors in these regions to block these changes.

Adult, male rats were treated for 10 days with PROG (0, 5 or 20 mg/kg s.c.) and/or HAL (0 or 1 mg/kg s.c.). Other rats, with unilateral 6-OHDA lesions of the nigrostriatal bundle, were treated for 10 days with PROG (0, 10, 20 or 40 mg/kg s.c.). Four days after the last injection rats were injected with PROG (0 or 20 mg/kg s.c.) 40 mins prior to d-amphetamine (A). Chronic, but not acute, treatment with PROG enhanced A (1 mg/kg i.p.) induced locomotion, but did not alter A (1.25 mg/kg i.p.) induced rotation. The effect of PROG was not altered by chronic coadministration of HAL. Our results demonstrate the ability of PROG to increase A induced locomotion, an effect also seen after chronic neuroleptic treatment. Furthermore, the inability of PROG to alter rotation suggest that chronic PROG may exert some of its behavioral effects through the mesolimbic, rather than nigrostriatal, dopamine system.


A68552 is a CCK-7 analogue which we have shown to have nanomolar affinity for pancreatic CCK receptors but is more metabolically stable than is CCK. We used A68552 to investigate possible CCK/dopamine (DA) interactions in the acquisition or expression of environment specific conditioned locomotor activity (CLA) produced by d-amphetamine (Am). During 5 days of conditioning trials, male rats were injected with Am (0 or 2.5 mg/kg, i.p.) and A68552 (0 or 100 μg/kg, i.p.) before being placed in a locomotor activity (LAB) cage for 1 hr; controls received identical treatments after being removed from the boxes. On the test day, all animals were injected with either vehicle or A68552 1 hr prior to being placed in the A68552 into the A68552 and injected with A68552 (25 ng) into the NAcc produced an 18% increase in activity by itself but significantly attenuated (-36%) the PIC (50 ng, n=17) induced locomotor activity. These results show that CCK antagonizes the hyperlocomotor effects of endogenous DA, and suggest that endogenous CCK may be a functional antagonist of DA in the NAcc, a hypothesis which is consistent with conclusions from electrophysiological experiments.

423.4 CCK FACILITATES ACQUISITION OF A DISCRIMINATED AVOIDANCE RESPONSE. J. RODENHISER, D. GRIDER, H. SHUCK, AND E. QUINON. Dept. of Psychology, University of Louisville, Louisville, Ky, 40292.

The octapeptide fragment of cholecystokinin (CCK 26-33) seems to modulate a wide range of behaviors, but the data are inconsistent. We have previously shown that CCK facilitates the acquisition of passive avoidance. The present study seeks to determine whether exogenous administration of CCK can facilitate the acquisition of a multi-trial discrimination task. C57BL/6J mice were given injections of either saline or 200 μg/kg of CCK-8 subcutaneously 30 minutes before training (7 trials/session, 8 sessions). The task required the animal to discriminate an illuminated door from among 5 doors, and to exit through the illuminated door to escape/avoid shock. Attempts to exit through non-illuminated doors were counted as errors. Error reduction was significantly greater in the CCK group over the entire sessions, although both groups were responding similarly over the last few sessions. These results suggest that CCK facilitates the acquisition of this task, and further supports the role of CCK in learning.

Suppression of food intake by cholecystokinin (CCK) is a vagally mediated behavior that is abolished in rats pretreated with the sensory neurotoxin, capsaicin. We have observed that suppression of food intake by intraperitoneal CCK (4 ug/kg) is associated with reduced motor activity (ai) in vehicle treated (VEH) rats. Reduction of body temperature by CCK did not occur in capsaicin pretreated (CAP) rats. (Table below.)

<table>
<thead>
<tr>
<th>TRT</th>
<th>MIN</th>
<th>RT °C</th>
<th>FOOD g</th>
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<tr>
<td>VEH</td>
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<td>0.0</td>
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<tr>
<td>CAP</td>
<td>60</td>
<td>40.0</td>
<td>40.0</td>
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(All means ± SE; **p<.02, RT significantly decreased; #p=0.05, food intake significantly reduced; *p=0.01, MIN, time post CCK or salin injection)

Additionally, there was no difference in CAP or VEH rectal temperature at 20 min post ip saline with no food present compared to 20 min with food present and consumed. This suggests that CCK-induced rectal temperature decrease can not be accounted for by decreased feeding activity alone. These findings indicate that CCK-induced reduction of body temperature is mediated by capsaicin sensitive neurons which may be similar or identical to capsaicin sensitive neurons involved in CCK-induced tachy.

Corticotropic releasing factor (CRF) is implicated in stress-related behaviors. Intracerebroventricular (icv) injection of CRF enhances the startle reflex (Swedlow et al., Psychopharmacology, 1986, 88, 147-152), but the size of action of this peptide has been limited. Previous findings indicate that the amygdala is involved in fear or shock-potentiation of startle, and that the spinal cord is involved in determining shock intensity in certain brain damage. Because CRF immunoreactivity and receptors are found in these areas, we investigated the effects of intracerebroventricular injections of CRF on startle as well as the effect of icv CRF on startle in amygdala-lesioned rats. Startle was elicited by 95 dB, 50ms noise bursts (510-530 background noise 55dB). Fifteen min after the first stimulus, artificial CSF or CRF was infused into the ventricle or lumbar spinal cord. CRF 0.5 or 3.0 µg injected icv or intracisternally produced a profound, dose-dependent enhancement of startle, whereas icv injection of 1.0 µg CRF produced a small increase. Lesions of the amygdala significantly ameliorated the effect of icv CRF (1.0 µg). The magnitude of enhancement produced by icv CRF in the amygdala-lesioned rats was comparable to that produced by intracerebroventricular injections of CRF in intact rats. These findings suggest that part of the icv CRF enhancement of startle may result from a direct action on spinal neurons and that the amygdala may be the primary site of CRF action or a critical part of the neural circuitry mediating icv CRF augmentation of startle. Experiments are in progress to investigate the effects of local CRF infusions into brain regions with CRF receptors.

424.7 DOES CRF CONTRIBUTE TO THE COGNITIVE CHANGES SEEN IN DEPRESSION? Z. Li, A. Malhi, J. Caloz, A. E. Barlett, Department of Neuropsychiatry, Research Institute of The John Howard Clinic, La Jolla, CA 92037

Recent studies have suggested that major affective episodes may be accompanied by cognitive impairment in some patients. In a positron emission tomographic study, utilizing event related potentials (ERPs) have also reported that some late component amplitudes (P2, P3) which are associated with cognitive events are also affected during depressions. One neurochemical factor which is known to be dysregulated in depression, and which may modify cognition is corticotropin releasing factor (CRF). In the present study we sought to evaluate the effects of CRF on ERPs in the rat in order to see whether CRF could produce changes in ERP morphology similar to those seen in human depression, using a two-chamber modified Y-maze paradigm. Eight male Wistar rats were chronically implanted with electrodes bilaterally placed in the frontopolar and dorsal hippocampus (DHPC). Rats were administered saline or CRF intracerebroventricularly (ICV) in doses of 0.0, 0.1, 0.5, and 1.0 µg per rat. The lowest dose of CRF (0.5 µg) produced significant reductions in the amplitude of the P2 component in cortex. Larger doses of CRF produced increased change in the P2 amplitude in cortex and significant dose dependent decreases in the P2 and P3 components in DHPC. These data are consistent with the idea that increases in CRF activity may contribute to the cognitive changes seen in depression. Supported by NASA 05096, 06059, and the MacArthur Fnd.

424.8 REVERSAL OF A NONMONOTONIC RELATIONSHIP BETWEEN FOOTSHOCK OR CRF AND THE ACOUSTIC STARTLE REFLEX BY BRIEF EXPOSURE TO A MILD STRESSOR. K. M. Barrett, K. C. Zhang, N. E. Barrett, Department of Psychology, Pharmacology, and Medicine, Univ. of Washington, Seattle, WA 98108

The neuropeptide vasopressin (AVP) has been implicated as a memory enhancing hormone in studies of passive and active avoidance. Previous studies administering AVP in a single dose intraventricularly have shown facilitated learning. Conversely, the centrally active AVP receptor antagonist d(CH2)5Tyr(Me)AVP impairs memory processes when injected acutely. In this study, we have examined the effect of chronic peripheral treatment with AVP or its antagonist on retention using a conditioned taste aversion (CTA) paradigm.

Male rats were chronically administered AVP, antagonist, or saline via an Accurel device implanted in the lateral ventricle. Two weeks after implantation the three groups were tested in a conditioned taste aversion paradigm for 3 weeks. AVP-antagonist-treated rats showed a more rapid extinction of aversions than the other two groups during the 2nd and 3rd weeks, indicating impaired memory for the aversion. There was no difference between AVP-treated and control rats. These results offer additional evidence for the involvement of AVP in learning and memory processes as measured by the CTA paradigm.

The present study examined whether AVP within the MPOA-AH is involved in controlling the inverse relationship between lordosis and flank marking normally observed during the estrous cycle. AVP, but not saline micro-injected into the MPOA-AH of ovariectomized (OVX) hamsters stimulated high levels of flank marking during tests with a sexually experienced male, but not saline micro-injected into the MPOA-AH of OVX hamsters. Stimulation of AVT in the MPOA-AH of OVX hamsters given E2 and progesterone (P) did not stimulate flank marking or inhibit lordosis during tests with a male. However, these same females exhibited high levels of flank marking in response to AVP when tested alone. A second experiment demonstrated that P was not required for inhibition of AVP induced flank marking in OVX females given E2 and tested with males. The present study provides evidence that AVP acts within the MPOA-AH to inhibit lordosis, but demonstrates that ovarian hormones and male social contact block the induction of flank marking by AVP microinjected into the MPOA-AH. These data suggest that one component in the neural coordination of lordosis and flank marking is the inhibition of the response of the MPOA-AH to AVP. (Supported by NSF BNS-8711373).


The impact of emotional stimuli on the reactivity of the immune system was studied in rats. To that end one trial learning passive avoidance test was chosen. As an in vivo immunological parameter a primary antibody response against Sheep Red Blood Cells (SRBC) (number of antibody forming cells (PFC)) was chosen. Exposure of rats to the passive avoidance apparatus resulted in an increase in primary antibody response (apparatus control group). A decrease in primary antibody response was demonstrated in rats that showed passive avoidance behavior; an inverse relationship between avoidance latencies and the magnitude of primary antibody response was observed.

Neuropeptides affect avoidance behavior. The effect of altered avoidance behavior, as a consequence of neuropeptide administration, on the immune response was studied. Microinjection of AVP facilitated avoidance behavior (avoidance latency control 62 sec vs DGAVP treated rats 300 sec). In DGAVP treated rats a decrease in number of PFC's was observed (control 930.10^6 PFC vs DGAVP treated rats 403.10^6 PFC). ACTH(4-10) also facilitated avoidance behavior (avoidance latency control 49 sec vs ACTH(4-10) treated rats 300 sec). In these rats however the inverse relationship between avoidance latency and number of PFC was disturbed (control 1300.10^6 PFC vs ACTH(4-10) treated rats 1900.10^6 PFC). CRF, intracerebroventricular administered, attenuated passive avoidance behavior (control 300 sec vs CRF treated rats 44 sec). In CRF treated rats an increase in number of PFC was observed (control 125.10^6 PFC vs CRF treated rats 245.10^6 PFC). The involvement of the autonomic system in modulating the immune response will be discussed.


It is known that the microinjection of arginine vasopressin (AVP) into the lateral septum, an area shown to have a high density of V1 receptors, has a pronounced effect on memory consolidation and temperature regulation in rats. The present study showed that microinjections of AVP into the lateral septum (LS), as well as the bed nucleus of the stria terminalis (BNST) were able to stimulate flank marking, a form of olfactory communication that functions to maintain dominant/subordinate relationships in hamsters. Microinjections of AVP (1ng/100nl) into the LS and BNST significantly elevated flank marking behavior, while microinjections outside these immediate areas were ineffective. In vivo receptor autoradiography, these data confirm that AVP stimulates flank marking in female hamsters, and suggests that the site of AVP action is similar to that in male hamsters. (Supported by NSF BNS-8711373).


Since the peptide AVT is a potent activator of oxytocin or angiotensin, into the LS and BNST and HD13508.)*

The present study examined whether AVP within the MPOA-AH is involved in controlling the inverse relationship between lordosis and flank marking normally observed during the estrous cycle. AVP, but not saline micro-injected into the MPOA-AH of ovariectomized (OVX) hamsters stimulated high levels of flank marking during tests with a sexually experienced male, but not saline micro-injected into the MPOA-AH of OVX hamsters. Stimulation of AVT in the MPOA-AH of OVX hamsters given E2 and progesterone (P) did not stimulate flank marking or inhibit lordosis during tests with a male. However, these same females exhibited high levels of flank marking in response to AVP when tested alone. A second experiment demonstrated that P was not required for inhibition of AVP induced flank marking in OVX females given E2 and tested with males. The present study provides evidence that AVP acts within the MPOA-AH to inhibit lordosis, but demonstrates that ovarian hormones and male social contact block the induction of flank marking by AVP microinjected into the MPOA-AH. These data suggest that one component in the neural coordination of lordosis and flank marking is the inhibition of the response of the MPOA-AH to AVP. (Supported by NSF BNS-8711373).

425.8 REGULATION OF BRAIN OXYTOCIN RECEPTOR BINDING BY ESTRADIOL AND BY PROGESTERONE. H. Coirini, M. Schuma,er and B.S. McEwen. The Rockefeller University, New York, N.Y. 10021.

We have previously shown that progesterone (P) increases the area of oxytocin (OT) receptor binding within the ventromedial hypothalamus in estrogen primed female rats by using the method of quantitative receptor autoradiography. Estradiol (E) induces OT binding within the ventromedial hypothalamus and surrounding area. P in turn causes the induced OT receptors to spread over OT fibers. By using the OT antagonist (i.e.) as a ligand, we recently found that the P-dependent spread of OT receptors is not limited to the ventromedial hypothalamus but that P treatment also causes a 50% increase in the area covered by OT receptors within different hormone sensitive brain regions. The spread of OT binding in both brain regions is dependent on the priming with estrogen and is rapid as it occurs within 4h of P administration.
425.9

BASAL FOREBRAIN FACILITATION OF LORDOSIS BY OXYTOCIN IS BLOCKED BY A UTEROTONIC ANTAGONIST. Caldwell, J.D., Barbata, A.S., Hardy, V.J., Smith, D.D. and Fedeler, C.A. BSRP and Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC 27599-7230.

In this study we compared the effects of equal doses of uterotonic, antidiuretic or vasopressin antagonist analogues in blocking the facilitative effects of simultaneous infusions of oxytocin (OXT). Ovariectomized estrogen-treated animals were infused through common bilateral cannulas in the basal forebrain with 500 ng OXT alone or in combination with a uterotonic, antidiuretic or a vasopressin antagonist. OXT significantly increased lordosis responding 20 and 40 minutes after its infusion into the medial preoptic area (MPOA) or anterior hypothalamus (AH) when compared with the response of normal saline vehicle infused animals. The uterotonic antagonist significantly blocked the facilitation seen after OXT. The vasopressor and antidiuretic antagonists had no effect on the OXT-induced facilitation of lordosis postures. The vasopressor antagonist facilitated sexual receptivity 90 minutes after infusion. This work confirms our earlier finding that OXT infusions into the MPOA-AH were facilitative to female sexual receptivity (Beh. Neurosci. 103:671). The facilitative effect of OXT on receptivity appears to be mediated by central uterine-like receptors which may have a separate CNS site from inhibitory vasopressor receptors.

425.10

SENSITIVITY OF BRAIN OXYTOCIN RECEPTORS TO OVARIAN STEROIDS. M. Schumacher, R. Corinna, D.W. Pfaff and B.S. McEwen. The Rockefeller University, New York.

In estrogen primed females, progesterone (P) increases the area covered by OT receptors within the ventromedial hypothalamus (VMH). This spread of OT receptors may be a key aspect to the facilitation of female mating as the infusion of OT into the VMH only facilitates lordosis behavior in female rats primed with both estradiol benzoate (EB) and P. In contrast, the facilitation of lordosis by OT receptors seems to be more efficient in the D phase than in the mid-light (L) phase of the light cycle. In this study we undertook a determination of the area covered by OT receptors and the endocrine changes correlated with the light cycle. To determine the area of OT that contributes to the facilitation of lordosis behavior, OT should be behaviorally more efficient during the D phase when the area covered by OT receptors is greatest. The infusion of a large amount of OT (1ug) into the third ventricle of EB and P primed unanesthetized rats facilitates lordosis behavior with the same efficiency during the L phase as well as the D phase. Hormonal administration of progesterone correlated with the light cycle in terms of changes in the number of OT receptors which correlated with changes in the number of OT sensitive areas.

REGULATION OF AUTONOMIC FUNCTIONS V

426.1

ALTERED NEURONAL CONTENT OF CALCITONIN GENE-RELATED PEPTIDE IN LAMINAE I AND II IN THE SPONTANEOUSLY HYPERTENSIVE RAT. K. N. Westlund, D. D. Disposable, O. B. Holland. The Marine Biomedical Institute and University of Texas Medical Branch, Galveston, TX 77550

Calcitonin gene-related peptide (CGRP), produced by alternative processing of the calcitonin gene, is a potent vasodilator. We have shown that dietary calcium deficiency significantly decreases the neuronal content of CGRP in lamina II and II of the dorsal horn of the spinal cord in the growing rat. To determine if the neuronal content of CGRP is altered in the spontaneously hypertensive rat (SHR, a model characterized by calcium deficiency, neuronal CGRP was localized immunocytochemically with high density of immunocytochemical staining was quantitated by computer-assisted image processing of laminae II and II of the upper thoracic spinal cord of 12-14 week old SHR (N=8) and WKy (N=8) normotensive, control rats. The SHR had significantly decreased neuronal CGRP content compared to the WKy rats (107±5 vs 12±6 arbitrary units, p<0.01). The neuronal density of substance P, which frequently co-exists with CGRP in this neuronal population, was not different between the two groups (SHR 916±6 vs WKy 883±3 arbitrary units). As expected, the SHR had a significantly higher tail-cuff systolic blood pressure than the WKy rats (21±10 vs 14±7, p<0.01; ±SEM). In conclusion, SHR have decreased neuronal content of CGRP which appears to be specific for CGRP since substance P was not altered. Therefore, this reduction of a potent vasodilator may contribute to the hypertension. Supported by NIH grant NS12255 and Am. Heart Assoc., TX Affiliate Grants 87R-654 and 88G-663.

426.2

PARABRACHIAL EFFERENTS INFLUENCE BOTH ANGIOTENSIN AND BLOOD PRESSURE SENSITIVE NEURONS IN AREA POSTREMA. S. Papaio and A.V. Ferguson (SPON:V.C. Abrahams) Dept.of Physiology, Queen's University, Kingston, Canada K7L 3N6

The area postrema (AP) is a circumventricular organ which has been strongly implicated in autonomic regulatory function. Previous electrophysiological studies have characterized subpopulations of AP neurons specifically influenced by systemic angiotensin II (AI) and angiotensin II (AII), or by changes in blood pressure (BP)-sensitive. We have also established that both angiotensin II and BP-sensitive neurons from the parabrachial nucleus (PBN). The present investigation was undertaken to determine the relationship between these groups of AP neurons and the endocrine changes correlated with the light cycle. To determine the area of OT that contributes to the facilitation of lordosis behavior, OT should be behaviorally more efficient during the D phase when the area covered by OT receptors is greatest. The infusion of a large amount of OT (1ug) into the third ventricle of EB and P primed unanesthetized rats facilitates lordosis behavior with the same efficiency during the L phase as well as the D phase. Hormonal administration of progesterone correlated with the light cycle in terms of changes in the number of OT receptors which correlated with changes in the number of OT sensitive areas.

426.3


The excitatory response of spinthalamic and spinotectal neurons to coronary artery occlusion (CAO) can be inhibited by stimulation of the raphespinal tract. The purpose of the present study was to determine if responses of spinthalamic and spinotectal neurons to CAO are similar. Twenty-three cats were examined at rest. Computer averages of SND were obtained in 45 VLM neurons in intact and barodenervated cats. These results suggest that bursts of SND slow wave activity occurred during the D phase. Thus, behavioral and endocrine changes correlated with the light cycle could interact with the OT effect.

426.4

A ROLE FOR SYMPATHOEXCITATORY VENTROLATERAL MEDULLARY NEURONS IN THE PRESSOR REFLEX TO MUSCULAR CONTRACTION. R.M. Bauer, G.A. Iwamoto and T.G. Waldrop, Departments of Physiology & Biophysics and Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801.

Studies from this laboratory have shown that the pressor reflex to muscular contraction (MC) is mediated by a sympathoexcitatory neuronal system in the rostral ventrolateral medulla (VLm). The present study was undertaken to determine if the firing patterns of VMN neurons are altered during MC. The purpose of the present study was to define the firing patterns of VMN neurons which are altered during MC. The firing patterns of VMN neurons which are altered during MC. The firing patterns of VMN neurons which are altered during MC. The firing patterns of VMN neurons which are altered during MC.

HURSDAY AM NEUROPEPTIDES AND BEHAVIOR: OXYTOCIN AND VASOPRESSIN 1071

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426.5 CYTOSKELETAL PROTEINS IN POSTGANGLIONIC NEURONS AND SIF CELLS IN THE CARDIAC PARASYMPATHETIC GANGLION OF NECTURUS. T.W. McKeon and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Two cell types are present in the cardiac ganglion of Necturus maculosus: postganglionic parasympathetic neurons (principal cells) and small-intensity-fluorescent (SIF) cells. Principal cells have ovoid nuclei and are "ladder-like" whereas SIF cells have several processes. The present study was done to determine whether processes of these cell types contained axonal or dendritic cytoskeletal proteins. Deaffrented cardiac ganglia were labeled using immunofluorescence techniques with antibodies for either the high molecular weight forms of microtubule associated protein-2 (MAP2; a gift from L.I. Binder) or for the phosphorylated form of the H and M subunits of neurofilament protein (SMI31). MAP2 has been shown previously to label cell bodies and dendrites, whereas SMI31 preferentially labels axons. Identification of SIF cells was confirmed by double labeling with a serotonin antibody. In control and deaffrented cardiac ganglia, principal cell somas and several hundred microns of their processes were labeled with MAP2, but SIF cells and processes did not label. SMI31 labeled many axons in the cardiac ganglion but did not label the principal cell somas or their proximal processes. Following deaffrentation, principal cell somas and proximal processes labeled with SMI31. SIF cells and their processes were not labeled with SMI31 in control or deaffrented ganglia. These results indicate (1) SIF cells in the medullary cardiac ganglion do not contain the same cytoskeletal proteins as the principal cells, (2) the proximal part of the principal cell process contains dendrite-like processes, and (3) deaffrentation promotes expression of phosphorylated forms of neurofilaments in parasympathetic postganglionic neurons. Supported by NIH NS 29378 and NS 29573.


Activation of the posterior hypothalamus (PH) of the rat induces lassitude, tachycardia, hyperventilation, hyper-vasodilation, and vasodilation within skeletal muscle. These changes consistent with activating the "central command" for exercise. The purpose of this study was to determine the mechanism by which PH activation induces this microvascular dilation. Male Sprague-Dawley rats (190-290 gm) were anesthetized with pentobarbital (50mg/kg, ip) and the cremaster muscle was suspended in a tissue bath of Kreuger solution. Articloles (12-24u) were observed by television microscopy while diaphragmatic ECG activity, blood pressure and heart rate were monitored. Phentolamine Methiodide (250μg/50nl) was microinjected into the PH before and after local blockade of β-adrenergic receptors (nicardipine) and substance-P receptors (SP6). To eliminate reflex dilation due to respiratory changes and/or local vasodilator metabolites from lassitude PH activation, animals (n=8) were paralyzed prior to the "central command" for exercise in the rat is due to the withdrawal of sympathetic tone from the microcirculation. Supported by Kentucky and Amer. Heart Assoc.


Coordination between physiological measures has been suggested to be an index of physiologic maturity in infants. We examined the developmental patterns of correlations between physiological measures in full-term infants over and Department of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90024.

23 normal full-term infants at 1 wk and at 1, 2, 3, 4, and 6 mo of age. MATURATION OF CORRELATIONS BETWEEN PHYSIOLOGICAL coordiations were examined visually and assessed by 2-way (age x state) analysis of variance. Different developmental patterns were observed in each sleep-waking state. In quiet sleep, the correlations weakened over the first mo. During waking, all correlations increased from birth to 2 mo and respiratory variability, and respiratory rate and respiratory variability during REM sleep were significantly correlated with mature motor activity (N=55) over and Department of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90024.


The upper respiratory tract (URT) serves as the first line of defense against inhaled irritants and is innervated by the ethmoidal, glossofaryngeal (X), and superior lar­yngeal (SLN) nerves. The primary afferent projections of these nerves to the trigeminal sensory complex were studied using the transganglionic transport of a mix of HRP solu­tions injected into the respective peripheral nerves. The ethmoidal nerve projected densely into ventrolateral laminae I and V of the rostral mediobulbar dorsal horn (MDH), the ventrolateral paraganglionic nucleus (VP), and ventromedial parts of the subnucleus interpolaris (TrSi), oralis (TrSo), and the principal nucleus (PV). A more sparse projection to the dorsolateral part of the Sl and PV. The SLN also projects to lamina I&V of the MDH and the ventral and dorsal parts of the ParaVa; a sparse projection to the ventral and lateral parts of the ParaVa; a sparse projection to the mediolateral edge of TrSi was noted. In addition, the nucleus solitarius was labeled in SLN and IX cases, and the reticular formation in all cases. Thus, afferent fibers from the URT have convergent projections to lamina I&V of the MDH and the ParaVa; secondary neurons in these areas may be important for the cardiorespiratory adjustments seen after URT stimulation. Supported by NIMH: HL38471.


Binding sites for the endothelin derived peptide,endo­thelin, were recently described in the rat brain. More­over, modified cerebral functions were reported upon icv administration. In order to investigate the respiratory effects of endothelin icv, we measured changes of ventilation rate (VR) and relative tidal volume (VT) in conscious, unrestrained Sprague-Dawley rats after icv doses of endothelin 3. Prior to the experiment, rats were implanted over the right parietal cortex (AP-0.8, L-1.2) under halothane anesthesia. The animals were allowed to recover for at least 24 hours before measurement in the Oxygater system (Columbus Instr.) after icv injections of 0.1, 0.3, 1, and 2 nmol/kg doses of endothelin 3 in a volume of 15 μl. The integrals of the changes were calculated and used for statistical evaluation. While VR in all groups was not significantly different from control (saline), RV, increased dose dependently by 50%, 129%, 263% (p<.005), and 319% (p<.005) compared to saline, leading to a dose dependent increase of the relative ventilation minute volume which was calculated with the formula: (RV(x100)/VT). These results indicate that endothelin stimulates ventilation and may imply a role for endothelin in the central cardioventilatory system.


The role of the CNS in control of airway smooth muscle tone is poorly defined. In fact, there are no well defined sites that govern bronchomotor tone have not been identified. To investigate the role of the medulla on airway smooth muscle tone we measured changes of blood pressure (IPM) following bilateral electrical stimulation of the medulla in anesthetized ventilated guinea pigs. Blood pressure was measured to determine cardiovascular responses to CNS stimulation. Stimulation of the medulla (10s, 32 Hz, 50-700 μA) elicited an intensity-dependent increase in IPM and hypertension. The greatest increase in IPM (587±19% at 700μA) occurred at: A 1.5-3.0 mm, L 1 and 10 µg/kg iv). Neither vagotomy nor i.p intrapleural blocked the hypertension due to CNS-stimulation. In contrast, spinal section with lidocaine abolished the hypertension but not the increase in PIP. These results identify a bronchoconstrictor site in the medulla, which projects to the long thoracic paravertebral pathway. It is speculated that this medullary bronchomotor area is an important site of convergence for reflex and supramedullary bronchomotor pathways in the brain.
426.11 OSCILLATIONS IN BREATHING PRESSURE OR FLOW RECOILS DUE TO ALWAYS SMOOTH NERVE ACTIVITY. J. Garcia Ramirez, Lab of europhysiology, University of Queretaro, Mexico. Breathing pressure or flow oscillations can be recorded in the conscious rat or anesthetized rat and dog by using ultrasonic detectors. These records may be caused by events taking place in upper air or extrathoracic orifices (heart, muscles) or be artifacts produced by the recording system. Experimental observations were made to exclude or control such possibilities. It was found that the irregular respiratory activities are randomly distributed in the thoracic cavity and depend from sympathetic activity. They are exaggerated by a deep inspiration, the Valsalva maneuver, curtail orification, cold or warm stimulation, cigarette smoking, moderate anaphylaxis, and emotional situations in man. Conditions when sympathetic activity are detected in other organs (p.27, superficial veins, heart rate, nictitating membrane). Furthermore, these oscillations appear in animals with the chest open, persist after the double vagotomy, but disappear after removal of both stellate ganglia with part of the thoracic chain. It is inferred that these oscillations represent physiological activity and it is related to events occurring in bronchial tissues. That activity depends from sympathetic action.

426.12 MECHANISMS OF THE ACTION OF ACETYLCOLINE ON GLOMERULAR ULTRAFILTRATION IN THE RABBIT. K. LUM (SPON: Ton M. V. Wang, Department of Medicine, N. R. Ohio College of Medicine, Rootstown, OH 44272). Acetylcolline was injected into the renal artery to examine its effect on the glomerular ultrafiltration and renal autoregulation in rabbits. First, acetylcolline injection produced variable changes: the renal blood flow (RBF) increased greatly (40%) and the ultrafiltration coefficient (Kf) decreased dramatically (38%). Both afterload and different arteriolar resistance (RAa RE) decreased. Because of these offsetting effects, glomerular filtration rate (GFR) was unchanged. Then McIl was injected and the contraction of mesangial cells was blocked. In such conditions, the injection of acetylcolline failed to reduce RBF, but RBF, RA, RE had the similar changes as before. Moreover, GFR increased significantly (30%). These data gave direct demonstration that Kf is a key factor of differential autoregulation of renal blood flow and glomerular filtration rate.

EPILEPSY: BENZODIAZEPINES AND INHIBITORY AMINO ACIDS

427.1 CENTRAL BENZODIAZEPINE RECEPTOR CHANGES IN THE p,p'-DDT MYOCINIC/EPILEPTIC RABBIT HIPPOCAMPUS. R.R. Trifiletti and M.R. Pranzatelli. Departments of Neurology and Pediatrics, Columbia University, New York, NY 10032. Certain benzodiazepines (BDZs) have anti-myoclonic properties in human myoclonic disorders and in the p,p'-DDT myoclonic/epileptic models in the rat. To test the hypothesis that BDZ receptor abnormalities may contribute to the neurotoxicity of p,p'-DDT, we measured central BDZ receptors in adult rats given myocastatic intubation using in vitro [3H]-flunitrazepam binding. The hippocampus was chosen for initial study since it contains both BDZ receptor subtypes. Animals treated with p,p'-DDT displayed a 23% (p<0.05) decrease in number of BDZ receptors compared to controls. Pretreatment of animals with intracerebral 5,7-dihydroxytryptamine (5,7-DHT), which markedly reduces the density of BDZ receptors, in the hippocampus was injected and the contraction of mesangial cells was blocked. In such conditions, the injection of acetylcolline failed to produce the same effects as before. Moreover, GFR increased significantly (30%). These data gave direct demonstration that Kf is a key factor of differential autoregulation of renal blood flow and glomerular filtration rate.

427.2 THE BENZODIAZEPINE (BDZ) RECEPTOR IN CULTURED ASTROCYTES FROM GENETICALLY EPILEPSY-PRONE RATS (GEPR-9). M.D. Norenberg and L. Duquet (SPON: R. Rotundo). Lab of Neuropathology, Veterans Adm. Med. Ctr. and Univ. Miami Sch. Med./Jackson Memorial Hospital, Miami, FL 33101. The BDZ receptor has been strongly implicated in epileptogenesis (Paul and Skulnick, Science 202: 892, 1978; McNamara et al, Proc Natl Acad Sci 77:3029, 1980). It has generally been assumed that this receptor is the neuronal one which is associated with the non-FIGURE 1: (Top) Distribution of GAD activity in the superficial layers of the superior colliculus in kindled vs. non-kindled rats. *, p<0.05; **, p<0.01; ****, p<0.001. FIGURE 2: (Bottom) Distribution of GAD activity in the deep layers of the superior colliculus in kindled vs. non-kindled rats. *, p<0.05; **, p<0.01; ****, p<0.001. GAD activity was significantly increased in the deep layers of superior colliculi from GEPR-9 rats as compared to controls. No significant changes in adenyl cyclase activity were found. Our findings indicate that a significant reduction in peripheral-type BDZ receptor occurs in GEPR-9 astrocytes. Furthermore, these observations suggest that BDZ receptor changes described in other epilepsy models may also occur in astrocytes.

427.3 GLIAL GABA UPTAKE INHIBITORS: ANTICONVULSANT ACTIVITY IN CHEMICAL SEIZURE MODELS IN RATS. S.F. Gonzalez, B. Twichell, E.B. Harbaugh, P. Krogsaard-Larsen, and A. Schouenborg, Dept. of Surg. and Pharmacol., Dartmouth Medical School, Hanover, NH 03756 and PharmacoBios Res. Ctr., Copenhagen, Denmark. Evaluation of glial GABA uptake inhibitors as anticonvulsants has been hampered by their inability to cross the blood-brain barrier. We compared the anticonvulsant properties and neurotoxicity of intracerebroventricularly (i.c.v.) injected 5,6,7,8-tetrahydro-4H-isoxazo[4,5-d]azepin-3(0)-thione (THPO) and 4,5,6,7-tetrahydropyrimidin-2-thione (THPO) in acute models of chemical convulsion in rats. Maximal convulsive seizures were elicited by bolus i.v. injection of pentyleneetetrazol (PTZ, 25 mg/kg) or pentylenetetrazol and THPO (100-750 µg, i.c.v.). Seizure thresholds were evaluated using timed i.v. infusions of PTZ (10.2 mg/min). THPO and THPO increased seizure thresholds, and blocked the extensor component of maximal PTZ seizures, but did not increase PTZ seizure thresholds. THPO produced fewer deficits in motor function than did THPO. The ability of THPO and THPO to block convulsions in the pentylenetetrazol and 5,7-dihydroxytryptamine (5,7-DHT), which markedly reduces the density of BDZ receptors, in the hippocampus was chosen for initial study since it contains both BDZ receptor subtypes. Animals treated with p,p'-DDT displayed a 23% (p<0.05) decrease in number of BDZ receptors compared to controls. Pretreatment of animals with intracerebral 5,7-dihydroxytryptamine (5,7-DHT), which markedly reduces the density of BDZ receptors, in the hippocampus was injected and the contraction of mesangial cells was blocked. In such conditions, the injection of acetylcolline failed to produce the same effects as before. Moreover, GFR increased significantly (30%). These data gave direct demonstration that Kf is a key factor of differential autoregulation of renal blood flow and glomerular filtration rate.

GAD activity was significantly increased in the deep layers of the superficial colliculus from GEPR-9 rats as compared to controls. No significant changes in adenyl cyclase activity were found. Our findings indicate that a significant reduction in peripheral-type BDZ receptor occurs in GEPR-9 astrocytes. Furthermore, these observations suggest that BDZ receptor changes described in other epilepsy models may also occur in astrocytes.

427.4 BICUCULLINE KINDLING IN RATS INCREASES GAD ACTIVITY IN THE NIGROTECTAL TARGET AREA. M. Garrett, S.E. Buchel and K. Gate (SPON: J. A. Childs), Dept. of Pharmacology, Georgetown Univ. Sch. Medicine, Wash. D.C., 20007; and Maryland Psychiat. Res. Ctr. Baltimore, MD 21208. The integrity of the deep layers of the superior colliculus is required for protection against maximal electroshock seizure test. By means of a strategic application of the GABA agonist muscimol, suggesting that the GABAergic nigrotransicional projection may mediate this effect. We have investigated whether this projection is involved in regulating seizure susceptibility by measuring tailcued GAD activity after kindling. Adult male Sprague-Dawley rats were kindled by daily treatments (for 14 days) with bicuculline (BIC) given at 2 mg/kg per day and reducing the dose by 0.5 mg/kg every 4-5 days. After this period the threshold convulsive dose of BIC was <50% of that required in non-kindled (saline-treated) controls. Ten days after the last BIC treatment, the rats were sacrificed, and the superior colliculus was divided into the deep and superficial layers, bilaterally. These tissues were assayed radioenzimatically for GAD activity, and values for bilateral tissues averaged for individual rats. GAD activity was significantly increased in the deep layers of superior colliculus from kindled rats as compared to non-kindled rats (45±0.1, 100±0.1 µmol/mg protein/min) relative to non-kindled controls (37±0.1, 95±0.1 µmol/mg protein/min). However, there was no significant difference in GAD activity between superficial layers of superior colliculus from kindled (56±2) vs. non-kindled (56±2) rats (p>0.1). Thus, GAD activity is augmented specifically in the nigrotransicional target area, and not in the superficial layers of superior colliculus. These results support the hypothesis that reduced inhibition of nigrotransicional activity by substantia nigra, hence elevated tonic GAD activity, is a consequence of sensitization to BIC-induced convulsions. *Garrett & Gate, Exp. Neurol. 97:143, 1987, **Sims & Pitts, J. Neurochem. 17:607, 1970.
GENETIC VITAMIN B6 DEPENDENCY AS A DETERMINANT OF EPILEPTIC DIATHESIS
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Vitamin B6 (B6) dependency relative to deficiency is considered a significant determinant of epileptic diathesis and starting point for expanding neurotransmitter disorders. The experiments were carried out in specially developed syngenic epilepsy-prone (EP) and epilepsy-resistant (ER) inbred mice of both strains. A group of EP females was treated with B6 (10 mg/kg in drinking water) throughout the entire period of pregnancy and lactation; then offspring received the same treatment until their maturity. The treated offspring were compared with control rats of both strains, obtained from non-treated EP and ER strains. The results indicated that the treatment with B6 significantly altered the sensitivity of genetically vulnerable rats to experimental seizures. These findings suggest that abnormalities of B6 metabolism play a role in the pathophysiology of epilepsy and may provide a basis for the development of new therapeutic strategies.
427.11

determination of Lorazepam in Rat Brain Using Combined Technique of Liquid-Liquid Extraction, Solid-Phase Extraction and High-Performance Liquid Chromatography (HPLC)


Lorazepam (LZP), a 1,4-benzodiazepine is used in the treatment of status epilepticus. In order to study the pharmacokinetics of LZP entry into brain, we developed a method for quantitation of LZP in rat brain using HPLC. LZP was extracted from 100 ng of brain tissue, which was homogenized by ultrasonic disruption in 1 ml of 5% TCA plus 0.1 M Tris-HCl at pH 10.5. Type VIII alkaline protease was added to the homogenate and the mixture was incubated for 1 hr at 50°C. Toluene was added to extract the internal standard, chlordiazepoxide (CDX) served as internal standard. The organic phase was dried and the residue redissolved in methanol and washed against isooctane. The resultant extract was applied to a 1 μl C18-Bond Elute column and washed with 2 μl of water. LZP and CDX were eluted with 250 μl of 70% methanol solution. LZP and CDX were assayed using a Rainin C18-microbore column (250 X 4.6 mm ID) and monitored by a UV detector set at 240 nm. The mobile phase was methanol/0.025 M H3PO4 (66/34, V/V) and the flow-rate was maintained at 1 μl/min. LZP eluted at 7.9 min followed by CDX at 10.0 min. Plots of peak area versus concentrations were linear over the range of 20-200 ng LZP.

427.12

distribution of valproic acid in the rat brain

Thomas J. Hoepner, Rush Medical College, Chicago, IL 60612.

Valproic acid (VPA) is one of the most effective anticonvulsants available, yet its mechanism of action is unknown. The present studies were undertaken in the belief that determining the distribution of VPA in the brain would provide further evidence to identify the specific mechanisms responsible for its action. Radiolabeled valproic acid (as the sodium salt) was injected intravenously into anesthetized rats. One hour later (the time of maximal effectiveness of the drug) the animals were sacrificed and the brain removed and frozen. The distribution of VPA was determined in 100 μm thick coronal brain sections using autoradiography. VPA concentrated in the granular layer of the olfactory bulb with almost no accumulation elsewhere in the brain. The distribution was restricted to the medial and dorsal portions of the bulb (areas with notable concentration of GABA terminals and peripherial benzodiazepine receptors).

We have also compared the distribution of VPA in animals with and without perfusion with saline and fixative. Perfusion clears the isotope from the vasculature (where it binds to albumin), but leaves the high concentration in the olfactory bulbs.

The participation of the olfactory bulb may contain the long sought binding sites for VPA.

427.13

effects of GABA-related drugs in two types of paroxysmal activity


In previous experiments, we have shown that chronic intracortical infusions of GABA induced, upon withdrawal, the appearance of spontaneous paroxysmal activity. This "GABA withdrawal syndrome (GWS)", found in rats and baboons, has been assumed as a novel model of partial epilepsy (Brain Res., 442, 117, 1988).

Interested in elucidating the contribution of GABA receptor agonists and antagonists to the phenomena, we applied phaclofen, a new GABA antagonist, in rats showing a GWS. Intracortical microinjections (0.2 ul, 10 nM) of phaclofen failed to produce antiepileptic effects, while being effective in diminishing or arresting the paroxysmal activity induced by GABA. The new GABA receptor agonist, beta-fenfluramine (β-FEN), induced microspike formation along the neuritic shaft. This effect was maximally apparent within 30 min. The length of the neuritic extension was also decreased, but is probably reflective of the reduced area of the growth cone. This effect was maximal within 30 min. Further characterization studies are in progress.

427.14

steroid regulation of hippocampal nerve cell growth in culture

B.R. Johnson* and R.E. Brinton, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Steroid factors play a critical role in the development and adaptation of the nervous system (McGwen and Brinton, 1987). We have recently found that the metabolite of the steroid, 5α-pregnan-3α-ol-20-one (DHP) decreases nerve cell excitability in a GABA-dependent manner and protects against chemically induced seizures (Gee et al., 1988). The purpose of this study was to determine the effect of the steroid DHP, which decreases nerve cell excitability, and 17β-estradiol, which increases GABAergic excitability, upon nerve cell growth in culture. Hippocampal nerve cells were cultured from E18 rat pups and seeded in serum containing media. Following attachment, media was exchanged for serum free. Nerve cells treated with 30 nM DHP showed a rapid response to the steroid. Within 15 min following DHP exposure the number of microspikes along the neuritic shaft was reduced by 20% and by 30 min was reduced by 80%. In addition to the decrease in microspike number, the total area of the growth cone was reduced. The length of the neuritic extension was also decreased but was probably reflective of the reduced area of the growth cone. In contrast to the effect of DHP, estradiol (30 nM), induced microspike formation along the neuritic shaft. This effect was maximal apparent within 30 min. Further characterization studies are in progress.

428.1

stimulatory effect of prolactin on oxytocin secretion in the lactating rat


In lactating rats, stimulation of dopamine (DA) receptors blocks suckling-induced oxytocin (OT) release, while blockade of DA receptors significantly increases OT release. Further studies with selective agonists and antagonists demonstrated that stimulation of the D-2 receptor with (+)-7-PiRT HC1 (completely prevented suckling-induced OT release, while a D-1 agonist, R (+)-SKF-38393 HC1, was ineffective. The selective D-2 receptor antagonist domperidone blocked the inhibitory effect of the D-2 agonist, and blocked the excitatory effect of the DA agonist. We have also studied the effects of OT on brain DA receptors. To test whether DA acts within the neural lobe to suppress OP release, isolated stalk-neurointermediate lobes (sNIL) were perfused in Krebs-Ringer medium and stimulated electrically for 4 sec after 70 and 170 min of perfusion. Neither agent (1μM) affected basal or stimulated OT release, suggesting that DA does not act directly on the neural lobe to inhibit OT release. The stimulatory effect of OT on DA receptors was not secondary to their actions to prevent or increase, respectively, PRL release, and 2) that in lactating rats, PRL may exert a physiological effect to promote OT secretion.
428.3 BRAINSTEM ORIGINS OF SUBSTANCE P-IMMUNOREACTIVE PROJECTIONS TO NEUROSECRETORY CELL GROUPS IN THE PARAVentricULAR NUCLEUS. J.C. Bittencourt1, R. Benucci2 and E.E. Saatchi2.1 The Salk Institute, San Diego, CA 92138 and 2 Montreal Gen. Hosp., Montreal, Canada.

Anatomical and pharmacological evidence suggests a role for substance P (SP) in the control of vasopressin (AVP) secretion, but the origins of SP-immunoreactive (IR) projections to the paraventricular (PVH) and supraoptic nuclei of the hypothalamus have not yet been identified. Axonal transport, immunohistochemical, and lesion approaches were used to address this issue. The results indicate that: (1) SP-IR varicosities are distributed in the PVH and preferentially to aspects of the magnocellular neurosecretory system in which AVP cells are concentrated. (2) Combined retrograde and immunohistochemical studies identified SP-IR in subsets of A1 and C1 catecholamine neurons that project to the PVH. The only other prominent brainstem contribution to the SP-IR projection arose from the laterodorsal tegmental nucleus (LDT), including some cholinergic cells. (3) Unilateral knife cuts at the level of the facial motor nuclei, which resulted in pronounced depletion of catecholaminergic varicosities in the PVH, also markedly reduced SP-IR, particularly in the magnocellular division. Coupled with the results of recent tracing studies, it appears that SP-IR inputs to the magnocellular system arise principally from the A1 catecholamine cell group. The LDT and C1 adrenergic cell groups appear to contribute to the parvocellular division of the paraventricular nucleus.

Supported by the NIH, the MRC and FAPESP (Brazil).

428.5 VASOPRESSIN SYSTEMS IN CHinese HAMStERS THAT HAVE DIABETES INSIPIDUS INDUCED BY MedROXYPROGESTERONE ACETATE. Juf de Vries, G.J. de Vries, G.C. Ferrara, J.J. Brewster1 and J. Coe2.1 Neuroscience and Behavior, Univ. of Massachusetts, Amherst; 2 Dept. of Physiology, Univ. of Massachusetts, Worcester; National Institute of Health, Hamilton, Montana.

Injections with medroxyprogesterone acetate (MPA) or Depo-Provera induce severe diabetes insipidus (DI) in Chinese Hamster (Cricetulus griseus) grizzlies lasting for over three months. Injections with pitresxin tannate temporally eliminate the DI (Goe and Ross, 1986). We studied the effects of MPA injections on (a) whether MPA induces changes in central VP systems, and (b) whether these changes are detectable via injection of antiserum to VP.

Hamsters were made DI with two injections of 5 mg MPA spaced one week apart. Controls received chloral hydrate or saline injection two weeks after the first injection, controls drank 5 ml per day or less whereas MPA-treated animals drank 29 ml per day or more. Animals perfused at that time showed SP a paraventricular (PVN) and supraoptic nucleus (SON) that stained equally well in brains of MPA-treated hamsters (n=5) and to be lower in VP immunocytochemistry. Radioimmunoassay showed no statistically significant differences in AVP levels in blood, pituitary, PVN and SON (n=5 vs per group). As they tended to be higher in the MPA-treated animals, MPA does not seem to inactivate VP neurosecretion. (Supported by NSF grant BNS-809799 to GJD and NIH grant NS-23557 to CPF)


Vasopressin (VP) cells in the bed nucleus of the stria terminalis (BST) medial amygdaloid nucleus (AME), supraoptic (SON) and paraventricular (PVN) nuclei are influenced by gonadal steroids. The goal of the present paper was to examine whether VP cells in the BST, AME, SON and PVN contain estrogen receptors. Brains from adult short-term castrated, colchicine-treated male rats were fixed for 8 hrs in 4% PAF and 0.5% glutaraldehyde. In the immunocytochemical double staining procedure vesicle sizes were first incubated with estrogen receptor antibody (M222, Abbott, Chicago) and stained with DAB-NiCl. Following a methanol-H2O wash, sections were then incubated with anti-VP-neurophysin and stained with DAB. Parvocellular cells in the BST and AME were double stained with a blue-black nucleus (indicating the estrogen receptor) surrounded by brown cytoplasm (resulting from VP-neurophysin immunoreactivity). Our results provide the first direct anatomical evidence supporting the hypothesis that gonadal steroids influence on paraventricular VP cells in the BST and AME is mediated directly via estrogen receptors localized in nuclei of VP neurons. We were unable to localize any estrogen receptors in magnocellular VP and oxytocin cells in the SON and PVN, suggesting that estrogen indirectly impacts magnocellular hypothalamic cells.

428.7 NEURAL LOBE EXTRACELLULAR SPACE AND ITS DEPENDENCY ON EXTRACELLULAR FLUID POTASSIUM (OR SODIUM?) AS OBSERVED AFTER ULTRA-RAPID FREEZING. M. Tian. J. Reger* and W.E. Armstrong. Department of Pharmacology, West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901.

The magnocellular neurons of the hypothalamo-neurohypophysial system (HNS) may contain opioid peptides such as methionine enkephalin (MENK) and dynorphin I-8 (DYN) as well as oxytocin (OT) and vasopressin (AVP). The opioids may provide feedback control over the OT and AVP release. If the opioids have such a functional role, the content or release of the opioids might be expected to change under conditions in which OT or AVP change. To examine this, the neural lobe extracellular space of the HNS release of the four peptides were determined under conditions in which OT is known to change. Diets, diethylthiocarbonyl (DDC)-treated, and day 22 pregnant rats were decapitated, the HNS excised and superfused in oxygenated Kbic sock at 31°C. Following superfusion, nonintermediate lobes (NIL) were removed and homogenized. Peptide content of superates and homogenates were determined by specific RIAs. NIL OT was increased in DES-treated and pregnant rats. AVP and DYN content were decreased in DES-treated and pregnant rats. Release of the peptides from the HNS paralleled changes in NIL content. These data are consistent with a functional role for the co-localized opioids in the control of OT and AVP release. Supported by NIH Grant HD 22562 and a WSOM Intramural Research Grant.


The purpose of the present study was to determine whether exposure to progesterone (P) regulates oxytocin (OX) mRNA levels in the paraventricular (PVN) and supraoptic (SON) nuclei.

Ovariectomized female rats were implanted with 2 mm Silastic capsules containing estradiol (E), and injected with either P (4 mg) or oil vehicle 24 hrs later. The SON was isolated from the PVN and SON using a small scale guanidine thiocyanate/OCl method. Oxytocin mRNA levels were measured using hybridization-RNase protection assay. The probe was a 110 base antisense RNA transcript from linearized plasmid pGEM-OXYC (a gift of Dr. Thomas G. Sherman, Univ. of Michigan).

No effect of P was detected in the SON. In the PVN however, P significantly increased OXY mRNA levels relative to E alone. These results indicate that long term exposure to high levels of P, in the presence of E, decreases OXY mRNA levels in the PVN.
428.9

ISOPROTERENOL ATTENUATES α2 ADRENERGIC RECEPTOR EVOKED VASOPRESSIN (VP) RELEASE FROM PERFUSED RAT HYPOPHYSOCITY IN THE PUPITARY STALK, J.R. Doherty, J.E. Hoffmam and C.D. Verbéii. (Dept. of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.) Effects of isoproterenol on VP release from HNS explants were studied. AD-receptor activation suppressed VP release in this model. The cisternum was not removed and the HNS explant was placed on a thermally insulating stereotactic device. VP release was measured at 10 min intervals for 60 min. The cisternum was removed, and the HNS explant was then placed in 25% (350 mosm/kg H2O) saline, and saline was then incubated with 10-4 M isoproterenol (IP) for 15 min. Isoproterenol significantly decreased VP release by 53% (p<0.05). This supports the hypothesis that AD-receptor activation suppresses VP release. (Supported by MRC and FRSQ.)

428.11

FUNCTIONAL NEUROHYPOPHYSIAL INDUCED BY CONTROLLED COMPRESSION OF THE PITUITARY STALK, J.R. Doherty, J.E. Hoffman and C.D. Verbeii. (Dept. of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.) Selective lesions of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) were produced in rats. The SON and PVN were ablated stereotactically and the rats were killed 2 weeks later. VP release was then measured in HNS explants. The SON and PVN explants released significantly more VP than the PVN explants. This suggests that the SON and PVN contribute to VP release in this model. (Supported by MRC and FRSQ.)

428.12

DENERVATION OF THE RAT POSTERIOR PITUITARY: VALIDATION OF A NOVEL SURGICAL APPROACH. G.B. Makra, Inst. Expd. Mod. Hung. Acad. Sci., H-1450 Budapest, Hungary. In rats, the pituitary stalk was transected using a Halban-type rotating knife. The rats were killed 1 week after surgery. VP release was then measured in HNS explants. The VP release was significantly decreased in the denervated rats compared to the control rats. This suggests that denervation of the pituitary stalk decreases VP release. (Supported by NIH RO1-DK-19761.)

428.13

EFFECT OF CHRONIC HYPOTENICITY ON BASAL AND OSMOTICALLY STIMULATED VASOPRESSIN RELEASE. C. Yagii and C.D. Sladek (SPON: M.T. Baurat). University of Rochester School of Medicine, Rochester, NY 14622. Effect of chronic hypotonicity on VP release from HNS explants was studied. Basal VP release was measured in HNS explants. The HNS explants were then placed in 50% (200 mosm/kg H2O) saline for 24 h. Basal VP release was then measured in the HNS explants. Basal VP release was significantly decreased in the hypotonic saline compared to the control saline. This suggests that chronic hypotonicity decreases VP release. (Supported by NIH RO1-DK-19761.)

428.14

EVIDENCE FOR INVOLVEMENT OF EXCITATORY AMINO ACIDS IN OSMOTIC STIMULATION OF VASOPRESSIN RELEASE. C.D. Sladek, M. Gallagher, and C. Yagii. Departments of Neurology and Neurobiology, University of Rochester School of Medicine, Rochester, NY 14622. Osmotic stimulation of VP release is abnormal in rats following electrolytic lesions of the region anterior and ventral to the anterior third ventricle. This suggests that osmoreceptors in this region are involved in the regulation of VP release. In the present experiments, we examined the possibility that excitatory amino acids (EAA) are involved in this pathway. To examine the effect of KA on VP release, the rats were anesthetized and the HNS explants were isolated. The HNS explants were then placed in 25 mM KA and VP release was measured. KA significantly decreased VP release. This suggests that EAA are involved in the regulation of VP release. (Supported by NIH RO1-DK-19761.)
MICROSPHERES. J. T. Cunningham, R. Nissen, and
VISUALIZED WITH RETROGRADE TRANSPORT OF LABELED
The lamina terminalis region is a major input to
widely distributed while the projection from OVLT
was primarily ipsilateral. Bilateral SON
projections from these midline structures
represent a minority of their input to the SON.
(Supported by MRC and NIMH fellowship MH09766-
01X BPN-2)

INHIBITION OF RAT SUPRAOPTIC NUCLEUS (SON)
NEURONS BY HISTAMINE (HA) H1-RECEPTOR ACTIVATION.
Univ. E. Lansing, MI 48824-1117
Histaminergic neurons of the posterior hypothalamus project to
the SON and excite vasopressin cells via H1-receptors. One
immunocytochemically confirmed and two putative oxytocin
neurons were reported (Soc. Neurosci. Abstr. 1988, 14:215) to be
hypo-polarized by HA. We investigated intracellularly recorded
responses of 24 SON neurons in hypothalamic slices to either bath
or nanodrop application of HA, H1, and H2 agonists and
antagonists. All putative or confirmed oxytocin cells were
inhibited (decreased firing) and/or hypo-polarized (by 5-20 mV) by
HA or the HA-agonist dimaprit. Cimetidine (H2-agonist) either
completely or partially reversed these effects. No effects on these
cells were seen with H1-agonists or antagonists. In one silent cell,
a nanodrop of 10^-12 M dimaprit produced a 20 mV hypo-polarization
that was reversed by a similar drop of cimetidine. This cell was
found to be vasopressinergic. Along with previous work, these
results suggest that histaminergic input to SON differentially
activates vasopressin and inhibits oxytocin neurons via H1 and
H2-receptors, respectively. Supported by NS 16942.

RESPONSES OF PLASMA ATRIAL NATRIURETIC PEPTIDE AND ARGinine
vasopressin to osmotic and volume stimulation. K. T.
Kalogeropoulou, A. M. Demirakou, L. G. Granger, E. P. Papadopoulou, M. I.
Harutyunyan, G. P. Dramadakis, and P. W. Gjedde (IPON, D. L. Weinshilboum, Clinical
Neuroendocrinology Branch, NIMH, and DEB, NICHD, NIH, Bethesda, MD 20892.
Arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) exert both
similar and dissimilar effects on salt and water metabolism. In studies of their
interactions, it has been shown that ANP inhibits vasopressin actions as well as
secretion, while AVP stimulates ANP release. These data suggest that ANP-AVP
interactions may work preferentially to facilitate protection from hypovolemia. To
further explore this question, we report here a study of the effects of 3% NaCl (given
as a rate of 0.1 ml/kg/min for 2.5 hrs) on simultaneous plasma ANP and AVP
collection. ANP and AVP concentrations, were measured at 30, 20, 10, and 10
min before infusion and every fifteen minutes, for 2.5 hours, thereafter. Eleven
healthy males and six healthy females (studied during both the early follicular and
late luteal phases) were included in the study. Hypertonic saline infusion induced a
6-fold increase in plasma AVP secretion from a basal value of 1.2±0.3 pg/ml to a peak
value 7.2±1.2 pg/ml, with a parallel 3-fold increase in plasma ANP from
49±7.7 to 166±15.7 pg/ml. Both the rise in plasma AVP and ANP correlated
significantly with the rise in plasma sodium, with a mean (±SEM) correlation
coefficient of 0.92±0.02 for AVP and 0.88±0.02 for ANP (p<0.001 for both).
No significant differences were observed in the correlation coefficient, the slope and
the osmotic threshold, between male and female subjects and between follicular and
luteal phase. The rise in plasma ANP correlated significantly with the rise in plasma AVP with a mean correlation coefficient of 0.84±0.03
(p<0.001) and a slope or sensitivity of 20.7±12.75 pg/ml ANP per pg/ml AVP. The
positive correlation between plasma AVP and ANP suggest that ANP and AVP
work together to correct hyperosmolality.

LAMINA TERMINALIS INPUT TO RAT SUPRAOPTIC NUCLEUS
VISUALIZED WITH RETROGRADE TRANSPORT OF LABLED
MICROSPHERES. J. T. Cunningham, R. Nissen, and
L. P. Renau. Center for Research in
Psych. & Neurosci. Michigan State Univ. E. Lansing, MI 48824-1117
Histaminergic neurons of the posterior hypothalamus project to
the TM and SON and excite vasopressin cells. These neurons are
being done to confirm the AP-TM projection. This is evidence for chemosensory and cardiovascular input to the
histaminergic cell group. Supported by NS 09140.
429.1

Control of proopiomelanocortin (POMC) gene expression is an important event in the body’s response to stress. Corticotropin releasing factor (CRF) and forskolin, which increase cellular cAMP levels, inhibit POMC gene transcription in the anterior pituitary by activating cAMP-dependent protein kinase. We are attempting to identify the near substrates of the kinase which might play a role in POMC gene regulation.

Using AIT-20 cells, a mouse anterior pituitary corticotropic tumor cell line, as a model for POMC gene regulation, we have identified several nuclear proteins which are phosphorylated in response to CRF and forskolin. Among these proteins is a 16 kd protein which appears to be tightly bound to a 9M urea-insoluble fraction of the nuclei and is only released from this fraction when it is sonicated. The phosphoprotein can be visualized on an SDS-PAGE gel by Coomassie blue staining, indicating its abundance in AIT-20 cell nuclei. Preliminary evidence indicates that this 16 kd protein might interact with DNA in the cell. The phosphoprotein has been partially purified by elution from SDS-PAGE gels and is currently being characterized using an in vitro phosphorylation assay. Future work will include examination of the ability of the phosphoprotein to bind to the 5’ promoter region of the POMC gene. Supported by NIH grant DK37407 and an American Heart Association grant-in-aid.

429.2

Calcium mobilization has been implicated in the sequence of steps that leads to the stimulated secretion of anterior pituitary hormones. Changes in the calcium/phosphorylation state of individual protein substrates is related to one of the transition events by which calcium ions modulate hormone release. Previous reports have documented that calcium/phospholipid- and calcium/calcium-dependent protein kinases stimulate protein phosphorylation in both anterior pituitary preparations. We have extended these investigations to evaluate the presence of calcium-dependent phosphorylase substrates in the rodent anterior pituitary.

Adult, male rats were decapitated, and the anterior pituitaries were harvested and homogenized. Aliquots were phosphorylated in the presence of 10 µM ATP (final concentration) containing [γ-32P]-ATP, along with either vehicle, calcium (10 mM), calmodulin (0.05 µM), phosphatidylserine (200 µg/ml), calcium/calmodulin, calcium/phosphatidylserine. The reaction was carried out for 1 min. The phosphorylated proteins were separated by 5% polyacrylamide gels and autoradiograms prepared. Densitometric analysis of the autoradiograms indicated the preferential phosphorylation of 54 and 74 KD proteins anterior pituitary proteins by calcium. The proteins of 66, 72, and 17 KD were preferentially phosphorylated in the presence of calmodulin or phosphatidylserine. Calmodulin stimulated the phosphorylation of a 19 KD protein. (Supported by DMIH R0-4288 and the N. United Way Fund.)

429.3

Nicotine(N)-stimulated ACTH release depends upon the activation of central mechanisms, particularly in areas accessible to N from the fourth (IV) ventricle. This region contains immunoreactive (CAT) nuclei and N stimulates the release of CATs. Moreover, CATs induce ACTH secretion via the release of hypothalamic CRF. Thus, the current studies determine whether central CATs are similar in N-stimulated ACTH secretion. 6-hydroxy-dopamine (6-OH-DA) or vehicle (SHAM) were injected into the lateral ventricle of rats; after 9 d, rats were given saline or 0.05 or 0.0 mg/kg bw i. N i.v. ACTH values (pg/ml) are as mean±SEM.

Saline: SHAM 24±6 21±4 20±3 18±3 19±3 46±2
6-OH-DA 32±9 42±11 34±7 27±4 44±10
0.03 N SHAM 17±6 19±7 24±14 185±51 13±26
6-OH-DA 11±5 15±12 57±4 46±5 53±8
0.05 N SHAM 29±5 29±65 43±154 352±178 167±99
6-OH-DA 32±7 127±29 84±25 61±12 48±39

In addition, the ACTH response to either stress was reduced from 62±69 (SHAM) to 37±27× (6-OH-DA) (p<0.05). This indicates that CATs may be involved as mediators of the ACTH response to nicotine. (Supported by DAMR77.)

429.4
MONOAMINE MEDIATION OF COCAINE-INDUCED HPA ACTIVATION. J.Borowsky and C.M.Kuhn. Department of Pharmacology, Duke University Medical Center, Durham, North Carolina, 27710.

The acute administration of cocaine (5-20 mg/kg,ip) to rats produced a dose-dependent elevation in both serum corticosterone (CS) and plasma adrenocorticotropin hormone (ACTH). These rises were maximal at 30 min and returned to basal values by 60 min. More selective DA uptake blockers GRB12909 and nomifensine also stimulated hypothalamic-pituitary-adrenal (HPA) axis activity, while the local anesthetic, procaine, did not. Pretreatment with haloperidol (0.2 mg/kg) significantly attenuated the increases in CS and ACTH elicited by cocaine, as well as the elevation in ACTH produced by GRB12909. Pretreatment with the D1 antagonist, SCH23390, or the 5HT2 antagonist, ketanserin, but not the a lpha -1 antagonist, prazosin, significantly decreased the ACTH rises following cocaine. Intracerebroventricular 6-OHDA lesions also significantly attenuated the ACTH response to cocaine. Following the repeated administration of cocaine (15 mg/kg, twice daily, 7 days), rats displayed a sensitized behavioral response to cocaine challenge while the HPA response to cocaine or saline was unaltered. The present results support a stimulatory role for dopamine, involving both D1 and D2 receptor subtypes, in regulation of HPA activity. However, both DA and 5HT could contribute to the adrenergic stimulation by cocaine.

429.5

We have previously reported that acute restraint stress results in ultrastuctural evidence for enhanced release of pro-opiomelanocortin (POMC)-derived peptides from the intermediate lobe (IL) of the pituitary (Carr et al., 1989, Anat. Rec. 233:224). In this study, we investigated the possibility that endogenous opioid peptides mediated peptide release from the IL during stress. Male Sprague-Dawley rats were injected with saline or one of two doses of the opiate-antagonist naltrexone (1 mg/kg or 10 mg/kg) 30 min prior to being placed in plexiglass restraint cages. Unrested rats received similar injections. Pituitaries were fixed at 2.5% glutaraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer for subsequent TEM. IL cells in unstressed animals contained numerous membrane-bound granules of various electron densities. As expected, there was a significant decrease in the granular content of IL cells in saline-treated rats stressed for 30 min. Pretreatment of rats with either 1 or 10 mg/kg naltrexone did not attenuate the degranulation of IL cells during stress. Conversely, naltrexone treatment appeared to potentiate the degranulation of IL cells of rats stressed with CRF. The naltrexone indicates that stress-induced release of POMC peptides from the IL is not mediated by opioid receptors. Supported by NIH NS21256 and RR08139 (LCS).
HYPOTHALAMIC-PITUITARY-ADRENAL DYSFUNCTION IS ASSOCIATED WITH COGNITIVE IMPAIRMENTS IN AGED RATS. A. Isa, S. Gauthier, W. Rowe, & M.J. Meaney. Douglas Hospital Research Ctr., Dept. of Psychiatry and Neurosciences, McGill Univ., Montreal H4H 1R3, Canada.

Increased activity of the hypothalamic-pituitary-adrenal (HPA) axis is associated with age-related neurodegeneration. The hippocampus, a critical brain region in learning and memory, is especially sensitive to glucocorticoids (GCs) and chronic exposure to elevated GCs results in the loss of hippocampal neurons (Seqel et al. J. Neurosci., 1985).

To assess the relationship between HPA activity and cognitive dysfunction in later life, we measured over 100 aged (24-29 month old) rats using the Morris swim maze. Three groups were compared: aged-handled (A), aged-handled (NH), and aged-impaired (IM). Aged IM rats had lower levels of corticosterone (4.8 ± 0.2 μg/ml) than A rats (6.0 ± 0.3 μg/ml), with the mediantime being 20%. In addition, A rats had lower levels of cortisol (11.0 ± 1.0 μg/ml) than IM rats (15.0 ± 1.0 μg/ml), with the mediantime being 20%. These data suggest that HPA dysfunction in the rat is associated with neural pathophysiology and does not occur merely as a function of age. Taked together with our previous studies (see Meaney et al. Science, 1988) these findings also suggest that individual differences in HPA function in later life are a major predictor of neuropathology in later life.

To determine the role of m-CPP in stimulating neuroendocrine function we compared the secretion of corticosterone, renin, and vasopressin in the rat. Groups of male rats (n=8) were injected with m-CPP (1 mg/kg, i.p.). Thirty minutes later rats were killed and trunk blood was collected and processed for radioimmunoassays of plasma corticosterone, renin, and vasopressin. Corticosterone was determined by the CAH interference assay. The dose of m-CPP was chosen as a low dose that evokes a measurable response. The dose of m-CPP was then increased by 1 mg/kg and the response was compared with that of the previous dose. The results showed that m-CPP stimulated the secretion of corticosterone and renin, but not vasopressin. The specific receptors involved and their stimulation mechanism are not well defined.


Increased synthesis of lipocortins (LC) is believed an important part of the anti-inflammatory effects of glucocorticoids. Western blot analysis of LC using specific antisera against LC I (resp. its 32 kDa fragment) and against LC II (Pepinsky et al., J. Biol. Chem. 261, 4280-4286) revealed that LC I and II levels were very low in rat brain and liver. LC I was specifically induced in cultured astrocytes. In contrast, LC II (or LC cross-reacting with antisera) was more prominent in microglia and was the predominant form in Kupffer cells. Hence, regulation of LC I/II synthesis is cell type specific.

429.16 ADRENOCORTICOTROPIN HYPERSECRETION ASSOCIATED WITH DIABETES MELLITUS RESULTIS FROM AN INCREASED RELEASE OF HYPOTHALAMIC-CORTICOTROPIN RELASING FACTOR (CRF) AND POMC. L. A. Johnson* and C. Casperman* College of Pharmacy, Wash. State Univ. Pullman, WA 99164-5100.

Adrenal hypertrophy and increased plasma levels of adrenocorticotropin (ACTH) and corticosterone have been observed in diabetes mellitus both experimentally and clinically. This study investigates the mechanisms responsible for this diabetes-associated hypersecretion of ACTH. It is not known. The present study examined the effects of experimentally-induced diabetes mellitus (streptozotocin [STZ], 65 mg/kg, i.v.) on the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic system. Diabetes mellitus (NPY), a 36 amino-acid peptide, is co-secreted with catecholamines during stress. We have shown that NPY activates APA at both the hypothalamic and the adrenal cortex by causing both ACTH and corticosterone release, respectively. To examine the hypothesis that the hyperthyroid status might affect the interactions between NPY and the APA axis, we studied chronically cannulated, freely moving male rats with long-standing (60 days) hypothryoidism, hypothyromysis, or euthyromysis. In all animals, we measured plasma concentrations of ACTH and corticosterone before and after an iv bolus of 10µg/100g BW NPY. Hypothyroid rats demonstrated significantly lower plasma ACTH and corticosterone concentrations after NPY administration than those of control rats. We conclude that hypothyroidism is associated with decreases in NPY-induced APA activation. The relative importance between the neuropeptide and the co-secreted catecholamines in regulating CRH secretion remains to be seen.
429.19 IN SITU HYBRIDIZATION OF VASOPRESSIN mRNA IN PARVOCELLULAR PARAVENTRICULAR (PVN) NEURONS IS ABOLISHED IN ADRENAL-ELECTROIZED (ADX) RATS BY LOW CORTICOSTERONE. S.F Akana, C.S. Caster*, and M.E. Dallman*. Univ. California Medical Center, San Francisco CA 94143-0444. ADX stimulates increases in CRF and vasopressin (VP) secretion and expression in parvocellular PVN, resulting in marked stimulation of ACTH secretion. Corticosterone (B) replacement of ADX rats restores ACTH to normal. To determine whether inhibition by B of ADX-associated increases in VP expression and secretion in parvocellular PVN is consistent with an inhibition mediated by high affinity type I (Kd 0.5 nM) or lower affinity type II (Kd 2.5-5nM) B receptors, ADX rats were replaced with 0.25 or 75% B pellets and killed in the AM on d 5. Blood and PVN brain sections were collected from these rats and sham controls; sections were hybridized with a 35S-labeled 33-base oligomer for AVP, emulsion-dipped, developed and examined for location of silver grains over parvocellular cells in PVN were observed in ADX, but were restricted to magnocellular cells in Shams and both B-treated ADX groups. Plasma ACTH was normal in the 5% B groups, but plasma B concentrations of 800mM total, or 1 mM estimated free B (Endo. 121:1194, 1987). We conclude that free B concentrations lower than the Kd for type II, but higher than the Kd for type I receptors are sufficient to normalize ADX-induced increases in AVP mRNA in parvocellular neurons of the PVN, suggesting a type-I mediated effect.

430.1 DENSITY OF SYNAPTIC INPUT IS IDENTICAL TO SMOOTH-CONTOURED AND THORNY GNRH NEURONS IN THE MALE RAT. L.W. Wilkin and K. Demaría*. Dept. Anatomy and Cell Biology, Columbia Univ. Coll. P&S, New York, N.Y. 10032. Gnrh neurons are typically smooth-contoured and fusiform in shape with a process extending at each pole. In the rat and other species, there is a subpopulation of these neurons which has an irregular contour with spiny projections from the cell soma and proximal processes. It is not known whether this difference is functionally significant, but the relative numbers of thorny Gnrh neurons is reduced in immature (Wray and Hoffman, Neuroend., 43:439, 1986) and castrate rats (Wilkin, Neuroend., in press). In order to discern whether the thorny neurons are more densely innervated (as has been suggested, Lemes et al., J. Comp. Neuro., 332:534, 1993), 4 adult male rats were deeply anesthetized with pentobarbital and perfused with paraformaldehyde/glutaraldehyde and brain sections from the preoptic area were treated immunochemically for the ultrastructural demonstration of Gnrh, using the LRI antibody (Bennet). 3 smooth and 5 thorny neurons were identified in 1 um sections and a series of ultrathin sections at 3 depths prepared. From photomicrographs of these cells, the percent of plasma membrane in synaptic contact was determined, and found to be the same in the two groups of neurons. To ascertain whether these cells might differ in some other aspects, the relative representation of a subpopulation of the two shapes were compared using point counting stereology. Smooth contoured neurons had more and larger nuclei but a smaller volume fraction of Golgi apparatus and mitochondria, while the representation of RER was similar in neurons of the two shapes. These results suggest that smooth contoured GnRH neurons are more actively transcribing message while thorny neurons are more actively engaged in peptide processing and packaging. These differences appear to be independent of synaptic input, but this must be confirmed by chemical identification of the synapses. NIH AG09066.

430.2 SEXUAL DIMORPHISM IN THE INNERVATION OF GNRH NEURONS IN THE RAT. W.P. Chen, J.W. Witkin and A.J. Silverman. Dept. Anat & Cell Biol., Columbia Univ. New York, N.Y. 10032. The pattern of gonadotropin secretion and presumably the release of Gnrh varies between the sexes. To determine if dimorphic neuroendocrine release might be due to differences in the synaptic input to Gnrh cells, we undertook a quantitative study identifying total input as well as two types of synaptic inputs, the dendroplasmic type-endorphin (B-E) and Gnrh. Young adult (3-4 mos) Fischer 344 rats (2 male and 4 female) were deeply anesthetized with sodium pentobarbital and perfused with paraformaldehyde/glutaraldehyde and brain sections from the preoptic area were treated immunochemically for the ultrastructural demonstration of Gnrh, using the LRI antibody (Bennet). 3 smooth and 5 thorny neurons were identified in 1 um sections and a series of ultrathin sections at 3 depths prepared. From photomicrographs of these cells, the percent of plasma membrane in synaptic contact was determined, and found to be the same in the two groups of neurons. To determine whether these cells might differ in some other aspects, the relative representation of a subpopulation of the two shapes were compared using point counting stereology. Smooth contoured neurons had more and larger nuclei but a smaller volume fraction of Golgi apparatus and mitochondria, while the representation of RER was similar in neurons of the two shapes. These results suggest that smooth contoured GnRH neurons are more actively transcribing message while thorny neurons are more actively engaged in peptide processing and packaging. These differences appear to be independent of synaptic input, but this must be confirmed by chemical identification of the synapses. NIH AG09066.

430.3 DIET RESTRICTION AND LHRH NEURON MATURATION. W.S. Lee*, and J.W. Witkin and A.J. Silverman. Dept. Anat & Cell Biol., Columbia Univ. New York, N.Y. 10032. CONTOURED AND THORNY GnRH NEURONS IN THE MALE RAT. W.P, Chen, J.W. Witkin and A.J. Silverman. Dept. Anatomy and Cell Biology, Columbia Univ. Coll. P&S, New York, N.Y. 10032. Neuroend.43:93.'86) and castrate rats (Witkin, Neuroend., in press). In immature rats, most Lhrh cells are characterized by immunoreactivity within the entire cell soma, while a subpopulation of these neurons which has an irregular contour with spiny projections from the cell soma and proximal processes. It is not known whether this difference is functionally significant, but the relative numbers of thorny GnRH neurons is reduced in immature (Wray and Hoffman, Neuroend., 43:439, 1986) and castrate rats (Wilkin, Neuroend., in press). In order to discern whether the thorny neurons are more densely innervated (as has been suggested, Lemes et al., J. Comp. Neuro., 332:534, 1993), 4 adult male rats were deeply anesthetized with pentobarbital and perfused with paraformaldehyde/glutaraldehyde and brain sections from the preoptic area were treated immunochemically for the ultrastructural demonstration of GnRH, using the LRI antibody (Bennet). 3 smooth and 5 thorny neurons were identified in 1 um sections and a series of ultrathin sections at 3 depths prepared. From photomicrographs of these cells, the percent of plasma membrane in synaptic contact was determined, and found to be the same in the two groups of neurons. To ascertain whether these cells might differ in some other aspects, the relative representation of a subpopulation of the two shapes were compared using point counting stereology. Smooth contoured neurons had more and larger nuclei but a smaller volume fraction of Golgi apparatus and mitochondria, while the representation of RER was similar in neurons of the two shapes. These results suggest that smooth contoured GnRH neurons are more actively transcribing message while thorny neurons are more actively engaged in peptide processing and packaging. These differences appear to be independent of synaptic input, but this must be confirmed by chemical identification of the synapses. NIH AG09066.

430.4 GNRH-, VASOPRESSIN-, AND GAD-IR NEUROENDOCRINE NEURONS INTERACT SYNAPTICALLY IN THE SUPRAOPTIC NUCLEUS OF JUVENILE MONKEYS. K.K. Thind, J.E. Bogser* and P.C. Goldsmith. Reproductive Endocrinology Center and Dept. of Ob/Gyn & Repro. Sci., Univ. Calif., Sch. Med., San Francisco, CA 94143. Vasopressin (VP) and gamma-aminobutyric acid (GABA) are hypophysiotropic hormones also involved in control of gonadotropin-releasing hormone (Gnrh) secretion. We examined whether Gnrh, VP, and glutamate acid decarboxylase (GAD) immunoreactive (-IR) elements interact synthetically in the supraoptic nucleus (SON) of cynomolgus monkeys. Neuroendocrine (NEU) neurons in 4 juveniles were retrogradely labeled with the retrograde tracer neurobiotin (NTH) to identify the SON and suprachiasmatic area. Long-axis sections of each were hybridized with the respective antibodies (VP with 5 nm gold, and GAD with 15 nm gold). Most of the VP-IR elements were located in the SON and only a few VP-IR elements were located in the suprachiasmatic area. Gnrh immunoreactivity with DAB and TMB as the respective chromogens was observed in the SON and approximately 45% in the suprachiasmatic area. Synaptic input is presumed to be due to the B-E input. China Medical Board (WPC), HD 10665 (AJS).

Previous studies from our laboratory suggest that the molar ratio of gonadotropin-releasing hormone-associated peptide (GAP) and LHRH in varicalis is similar in cell bodies, fiber varicosities, and nerve terminals during the estrous cycle of the rat. In the present study, we have evaluated these changes in peptide distribution and identity using radioimmunoassay and immunocytochemistry. Punched out from the medial preoptic nucleus (MPO), diagonal band of Broca (DBB), retrochiasmatic area (RCA), arcuate nucleus (AN) and median eminence (ME) were collected from 60 rats. Materials, that were separated according to molecular weight, were screened both for radioimmunoreactivity (RIA) with MC-2 antisera and for LHRH-LI with A772 and Rice #5 antisera. A concentration gradient of precursor to products was found from the cell body to the nerve terminal region(s). The MC-2 antisera detected materials in the MPO and DBB at approximately 14000-16000, 8500, and 6700 molecular weight, while the MC only contained the latter two. The distribution of different molecular forms of LHRH were bound in the MPO, DBB and RCA, while the AN and ME contained only mature LHRH. These data suggest that at least 3 additional post-translational steps must occur before the mature LHRH is produced from pro-LHRH. The regulation of these processing steps may have important functional consequences during the estrous cycle.


Pharmacologic evidence suggests that neurons which secrete GnRH are regulated by a wide variety of neurotransmitter systems. To unambiguously determine the neurochemical identity of synaptic inputs onto GnRH neurons in sheep, we used a double label immunocytochemical protocol which yields visibly different reaction products at both light and electron microscopic (EM) levels (Norgren & Lehman, 1987). Preparations of preoptic areas (POA) were perfused intracranially during mid-breeding season and potential contacts examined between immunoreactive tyrosine hydroxylase (TH), neuropeptide Y (NPY), substance P (SP), or glutamic acid decarboxylase (GAD) terminals and GnRH neurons in the preoptic area and anterior hypothalamus. At light and EM levels, NPY-, SP-, and GAD-positive varicosities were more frequently apposed to GnRH somas and dendrites than were TH-positive boutons. In all thin sections, non-identified terminals contacting GnRH neurons were more numerous than immunoreactive ones. TH-, NPY-, and SP-positive axon terminals contained clear spherical vesicles (20-40 nm dia.) and larger dense-core vesicles (80-120 nm dia.); GAD terminals contained only clear small vesicles. Thus far we have seen synaptic modifications only between NPY and SP terminals and GnRH dendrites. [Supported by NIH grants HD 18337 (FKJ) and HD 21968 (MLN)].


The presence of galavin (GAL) fibers and terminals in the median eminence of the hypothalamus suggests an involvement of GAL in neuroendocrine functions. GAL is broadly distributed and co-localized with several peptides and amines. The distribution of GAL terminals within the preoptic area (POA) is similar. Although the majority of GAL neurons are round and multipolar, a certain subpopulation of LHRH perikarya within the preoptic area (POA) is similar. Although the majority of GAL neurons are round and multipolar, a certain subpopulation of LHRH perikarya in the POA is also immunoreactive for GAL. Each of three major technical approaches, i.e., "adjacent section method," the "elution retaining technique" and the "direct double staining technique" indicated that a subpopulation of LHRH neurons in the POA co-express GAL. This represents the first demonstration of co-localization of another peptide in an LHRH neuron. Co-localization of GAL with LHRH may influence the release of LHRH; (b) may participate in the autoregulatory mechanism of LHRH secretion; (c) may be released and interact in a cooperative manner with LHRH on pituitary gonadotropes.

POSTNATAL DEVELOPMENT OF proGnRH-GAP mRNA in the rat preoptic area: anterior hypothalamic (POA-AH) was followed from postnatal day 15 through day 63. Sprague-Dawley rats were sacrificed by decapitation and cryopreservation; RNA was fractionated from individual hypothalamic homogenates and purified by use of proteinase K digestion. Cytoplasmic proGnRH-GAP mRNA was quantitated along with cyclophilin mRNA (an internal standard control) using solution hybridization-RNase protection assay. Cyclophilin mRNA levels normalized the content of total RNA provided to remain constant across the different age groups (p = 0.3). However, proGnRH-GAP mRNA showed a significant sex-related increase with age (p = 0.0001). In females, the mean proGnRH-GAP mRNA levels increased from 1.7, 3.0, 4.8, 3.7 and 3.8 on days 15, 20, 25, 29 and 34, respectively, to 7.2 and 15.5 on days 44 and 63, respectively. In males, the mean proGnRH-GAP mRNA levels increased from 2.3 and 3.3 on days 15 and 20, respectively, to 6.2-7.3 in the older age groups. These results show a sex difference in the postnatal development of proGnRH-GAP gene expression in the rat POA-AH. In males, proGnRH-GAP mRNA levels increase around the time of weaning (day 25) and remain unchanged thereafter. In females, proGnRH-GAP mRNA increases increase at the time of puberty (day 44) to levels shown by adult males, with a further 2-fold increase in adulthood.
430.11

**COMPUTER 3D RECONSTRUCTION OF MEDIAN Eminence MICROCAPILLARIES AT HIGH RESOLUTION.** L.S. Bibb, B.J. Dornes-Hartman, R.B. Page. *Washington University School of Medicine, St. Louis, MO 63110, and The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The microvascularity of the median eminence (ME) of the hypothalamus modulates hormonal communication between the brain and the pituitary. We are now reconstructing capillary microcapillaries within the ME in the aim of locating the cellular apparatus responsible for blood flow control. These loop structures were selected after extracting the 3D reconstruction of a low magnification study of this same tissue block, reported earlier (Soc. Neurosci. Abstr., 14:630, 1988). In summary, we found several capillary structures projecting from the outer to the inner plexus of the ME, like those observed in previous work (Page, et al., Am. J. Anat., 146:273, 1976).

We are now reconstructing discrete capillary structures with attached cellular elements from TEM images at 1650X. Digital image processing techniques are being used to create mosaics of overlapping images on each level, and to align the resulting mosaics (Bibb, et al., Comput. Bio. Res., 16:411, 1986). Edge detection and tracking on the mosaics will provide intercellular boundaries, and local texture measures may be used to denote intracellular contents. (Support: NSF BNS-8506749 and NIH NS15926.)

430.13

**GLUTAMATE IMMUNOREACTIVITY IN THE RAT NEURAL LOBE: AN ULTRASTRUCTURAL ANALYSIS OF NEUROSECRETORY ENDINGS AND PITOCYTES.** R.B. Meeker, D.J. Swanson, R.S. Greenwood and J.N. Havward. Neurobiology Curriculum and Dept, of Neurology, University of North Carolina, Chapel Hill, NC 27599.

To explore the possibility that the high level of glutamate immunoreactivity which we have previously observed in the neuroendocrine cell bodies of the supraoptic nucleus might represent a neurotransmitter pool of glutamate, we examined the distribution of glutamate immunoreactivity within the neuroendocrine neurons in the neural lobe. Cells fixed by perfusion with 4% paraformaldehyde, 2% glacial acetic acid and 15% saturated picric acid in 0.1 M cacodylate buffer were cut on a vibratome and stained with a rabbit polyclonal anti-glutamate-hemocyanin. Both pre- and postembedding (1:5000, ABC technique) and post-embedding (LR White, 1:10,000 Ab, 10-15 nm goat anti-rabbit colloidal gold) staining revealed a high level of glutamate immunoreactivity in the neuroendocrine neurons which was found in endothelial cells, phagocytic structures or astroglia with the hypothalamus. Neurosecretory axons or swellings rarely stained while a number of target terminals exhibited a high level of staining over the population of small clear vesicles. Two days of water deprivation increased the level of glutamate immunoreactivity in the pituicytes and neurosecretory endings. These observations suggest that glutamate might be used as a transmitter by the neurosecretory endings although the levels of glutamate are very low. Glutamate in the pituicytes may reflect a number of possible functions including: 1) uptake of extracellular glutamate, 2) neutralization of ammonia, 3) active GABA synthesis or 4) a specialized pool associated with local osmoregulation. (Supported by NHIJavits Award NS-14311.)

430.15

**HORMONAL CONTROL OF NPY IN THE SEXUALLY DIMORPHIC HYPOGASTRIC GANGLIA.** B. Schröder and R.W. Hamill.

We are now reconstructing discrete capillary structures with attached cellular elements from TEM images at 1650X. Digital image processing techniques are being used to create mosaics of overlapping images on each level, and to align the resulting mosaics (Bibb, et al., Comput. Bio. Res., 16:411, 1986). Edge detection and tracking on the mosaics will provide intercellular boundaries, and local texture measures may be used to denote intracellular contents. (Support: NSF BNS-8506749 and NIH NS15926.)

430.14


High concentrations of immunoreactive neuropeptide Y (NPY) varicosities exist in the paraventricular nucleus, paraventricular nucleus (PVN), hypothalamus (ME) and basal hypothalamus with lower levels in the supraoptic nucleus (SON). Within these regions NPY may function to control local blood flow (Evinrinov, et al., 1987) and/or regulate vasopressin secretion (Swanson, 1987). To more clearly understand the role of this peptide we examined the ultrastructure of the terminal fields in the SON, PVN and ME. The brain of male Sprague-Dawley rat was fixed by perfusion with 4% paraformaldehyde, 1% glutaraldehyde, 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.4. Vibratome sections were cut at 50 um and stained utilizing a polyclonal antiserum to neuropeptide Y (1:2000) and the double-bridge PAP technique. We observed a high density of large terminal endings containing clear round and dense-cored vesicles making axodendritic or axosomatic contacts. In the PVN as well as more modest levels of input to the SON. Within these regions NPY may function to control local blood flow and/or regulate vasopressin secretion. Haloperidol caused a significant fall in blood flow in the meatal region while the neurosecretory terminals exhibited a low level of staining with the neurosecretory terminals within the SON. Within these regions NPY may function to control local blood flow and/or regulate vasopressin secretion.

430.16

**TEMPORAL RELATIONSHIPS BETWEEN 17ß-ESTRODIOL, LUTEINIZING HORMONE AND GALANIN DURING SEXUAL MATURATION IN THE FEMALE RAT.** S.M. Gabriel. Departments of Psychiatry, Mount Sinai School of Medicine, New York, New York, NY 10029.

Recent studies suggest that the 21-α-amino acid peptide galanin is potentially regulated by estrogen in neuroendocrine tissues of the rat (Kaplan, Gabriel, Koening et al. PNAS 85:7409-7412, 1988). This estrogenic influence is first expressed during puberty when concentrations of galanin-like immunoreactivity (galanin-LI) increase in the anterior pituitary (AP), neurointermediate lobe (NIL) and median eminence (ME). PMSG injection causes a significant increase in galanin-LI concentrations in the ME, NIL and AP. The effect of estradiol on galanin-LI concentrations in the ME, NIL and AP was studied during this period of development. In ovariectomized female rats, galanin-LI concentrations are increased in the PVN as well as more modest levels of input to the SON. Within these regions NPY may function to control local blood flow and/or regulate vasopressin secretion.
430.17 GONADAL STEROIDS MODIFY DENTRIFIC SPINE IN HYPOTHALAMIC NEURONS: A GOLGI STUDY IN THE ADULT RAT. M. Franklin, E. Coulton. Neuroendocrinology Lab, The Rockefeller University, New York, NY 10021.

A golgi study of neurons in the adult rat ventromedial (VMH) and dorsomedial (DMH) hypothalamic nucleus was done in intact male and female rats and ovariectomized (OVX) rats treated with oil (O), estrogen (E) or estrogen and progesterone (P). Golgi-impregnated neurons were drawn and then analyzed using a computer for morphometric determination of differences in cell body size, number of primary dendrites, number of branchpoints and spine density. Statistical differences were determined using one-way ANOVA followed by the Newman-Keuls test (p<0.05). In the VMH of OVX rats given E alone or E in conjunction with P, there was a significantly greater density of dendritic spines than in the OVX-O group. There were no sex differences in any parameter of the VMH. In the DMH, the OVX-E-P group contained a significantly greater density of dendritic spines than the OVX-O group. Moreover, in the DMH of intact males there was a greater density of dendritic spines than in the intact females. There were no differences in cell body size, number of primary dendrites or number of branchpoints in either the VMH or DMH. From these results it appears that steroid feedback can result in short-term morphological changes in the hypothalamus.

Supported by N141576.

430.18 IMMUNOCHEMICAL LOCALIZATION OF AROMATASE IN THE QUAIL BRAIN. J. Olthuis and A. Foidart. Laboratory of General and Comparative Biochemistry, University of Lege Belgium.

The presence of aromatase in the brain of vertebrates was detected a long time ago. In many studies, the activity of the enzyme was studied by in vitro radioenzyme assays. These showed that the enzyme has a discrete localization in the brain and that its activity is modulated by the physiological condition of the animal. Factors such as sex, age, estrus, or steroid therapy all affect enzyme activity. However, the precise neuroanatomical localization of the enzyme and the mechanisms underlying the activity changes (modification of enzyme concentration or regulation of enzyme activity) have not been determined so far. By using a polyclonal antibody raised against human placental aromatase and immunocytochemical method using a polyclonal antibody raised against human placental aromatase and purified by affinity chromatography, we have for the first time successfully localized aromatase containing cells in the brain of a vertebrate, the Japanese quail. The specificity of the immunocytochemical reaction has been demonstrated. The cytoplasmic localization of the enzyme is confirmed. It is found primarily in neurons but double labelling experiments using specific neuronal or glial markers should be performed to confirm this conclusion. Aromatase positive cells are detected in all areas which were shown previously to contain aromatase activity by product formation assays, namely the preoptic area (POA) and several hypothalamic and septal nuclei. Within these areas, the distribution of the immunoreactive cells is however heterogeneous. In particular, within the POA most if not all positive cells are found in the sexually dimorphic preoptic medial nucleus (POM). In the POM, the number of immunoreactive cells and the intensity of their staining were drastically decreased by castration and restored to levels typical of sexually mature males by testosterone treatment. This demonstrates that the induction of aromatase activity which is caused by testosterone reflects real changes in enzyme concentration (enzyme induction) rather than a modulation in the activity of a constant number of molecules.


Although [endorphin (-endo) may play an important role in neuroendocrine function of the guinea pig, the distribution of -endo in the guinea pig brain has not been examined. Guinea pigs were etherized and one week later were injected with 25µg estradiol benzoate or oil. Animals were perfused 24h later with 4% paraformaldehyde. Brain sections (50µm) were cut on a vibratome. [3H]End was localized using an avidin-biotin immunocytochemistry (Einecke BE2 antisera). No cell bodies were seen rostral to the retrochiasmatic area (RCA). In the RCA, cells (10-50/section) were seen lying basomedially and extending laterally. In the rostral ARC, cells were concentrated (100-200/section) between the third ventricle (IVL) and appears that lateral feedback can result in short-term morphological changes in the hypothalamus.

431.1 EFFERENT CONNECTIONS OF THE ANTERIOR HYPOTHALAMIC AREA (AHA) STUDIED WITH THE NEW ANTEROGRADE TRACER PHA-L. J.M. Wilder and Th. van Groen, Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

The projections arising in the anterior hypothalamic area (AHA) have been characterized previously using the somatostatin method for detection of anterogradely transported [3H]amin acids. In the present experiments we have employed the PHA-L method to determine the specificity of these projections in more detail. The PHA-L method facilitates the distinction between fibers of passage and synapsing fibers. Iontophoretic injections were given in the mediobasal hypothalamus, the lateral hypothalamus, the ventromedial hypothalamus, the paraventricular nucleus of the thalamus, the central gray, and the nucleus substantia nigra.

In many studies, the activity of the enzyme was studied by in vitro radioenzyme assays. These showed that the enzyme has a discrete localization in the brain and that its activity is modulated by the physiological condition of the animal. Factors such as sex, age, estrus, or steroid therapy all affect enzyme activity. However, the precise neuroanatomical localization of the enzyme and the mechanisms underlying the activity changes (modification of enzyme concentration or regulation of enzyme activity) have not been determined so far. By using a polyclonal antibody raised against human placental aromatase and purified by affinity chromatography, we have for the first time successfully localized aromatase containing cells in the brain of a vertebrate, the Japanese quail. The specificity of the immunocytochemical reaction has been demonstrated. The cytoplasmic localization of the enzyme is confirmed. It is found primarily in neurons but double labelling experiments using specific neuronal or glial markers should be performed to confirm this conclusion. Aromatase positive cells are detected in all areas which were shown previously to contain aromatase activity by product formation assays, namely the preoptic area (POA) and several hypothalamic and septal nuclei. Within these areas, the distribution of the immunoreactive cells is however heterogeneous. In particular, within the POA most if not all positive cells are found in the sexually dimorphic preoptic medial nucleus (POM). In the POM, the number of immunoreactive cells and the intensity of their staining were drastically decreased by castration and restored to levels typical of sexually mature males by testosterone treatment. This demonstrates that the induction of aromatase activity which is caused by testosterone reflects real changes in enzyme concentration (enzyme induction) rather than a modulation in the activity of a constant number of molecules.

HYPOTHALAMUS

431.2 HYPOPHYSIAL TRACT: QUANTITATIVE COMPARISON IN 24 SPECIES. D. P. Sutherland,1 R. J. Rudo and R. S. Masterton, Department of Psychology, Florida State University, Tallahassee, FL 32306.

We have labeled the cell groups projecting to the spinal cord in 23 mammals and one reptile by histofluorescence of anterograde transport of FluoroRuby (F. K. Stephan). Dept. Psychology, Florida State University, Tallahassee, FL 32306.

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We have labeled the cell groups projecting to the spinal cord in 23 mammals and one reptile by histofluorescence of anterograde transport of FluoroRuby (F. K. Stephan). Dept. Psychology, Florida State University, Tallahassee, FL 32306.
431.3 PEPTIDERIC AFFERENTS TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. M. M. Meza and C. R. Johnson. Dept. of Physiol. and Neurosci., Univ. of Chicago, Chicago, IL 60637. 

The paraventricular nucleus of the hypothalamus (PVH) contains numerous cell groups that are immunoreactive for a variety of neuropeptides. To determine the significance of these peptides, it will be necessary to identify their origins. We examined the chemical specificity of forebrain afferents to the PVH using six neuropeptide antisera combined with fluorescent retrograde tracing. After Fluorogold injections into the PVH, small numbers of retrogradely labeled neurons were found throughout the hypothalamus, with a predominance of periventricular nuclei. 


We have previously reported that TGFα mRNA was expressed in the hypothalamic region of the rat brain during development. However, the role of TGFα in regulating LHRH release remains to be elucidated. In the present study, we examined whether TGFα mRNA is expressed in the hypothalamic region of the rat brain postnatally. 

431.5 DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST LHRH. H. F. Urban and J. A. Hackett. (SPON: C. L. Bethes). Div. of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006. 

Monoclonal antibodies (MAbs) against human LHRH were produced by fusing spleenocytes from BALB/c mice with SP2/0 myeloma cells and grown in HAT selective medium. The secreted MAbs were purified by precipitation with ammonium sulphate followed by affinity chromatography using Bakerbond AB2. Two of these LHRH-MAbs were subsequently characterized in detail. MAb HU4H showed conformational specificity to both mammalian and chicken LHRH, whilst MAb 11-111B showed sequential specificity to the C-terminal of mammalian but not chicken LHRH. Neither HU4H or 111-111B cross reacted with other mammalian hormones. 

431.6 ANGIOTENSIN (1-7) IMMUNOREACTIVITY IN THE RAT HYPOTHALAMUS. C. Block, H. Vilaseca*, and C. F. Ramirez. Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195-5070. 

We have recently reported the immunocytochemical (ICC) distribution of a novel heptapeptide (Ang(1-7)) in the rat brain. The present study was designed to analyze the possible involvement of Ang(1-7) in the regulation of hypothalamic functions. 


Cflow that the density and organization of immunoreactive axons that are immunoreactive for a variety of neuropeptides. To determine the significance of these inputs, it will be necessary to identify their origins. We examined the chemical specificity of forebrain afferents to the PVH using six neuropeptide antisera combined with fluorescent retrograde tracing. After Fluorogold injections into the PVH, small numbers of retrogradely labeled neurons were found throughout the hypothalamus, with a predominance of periventricular nuclei. 


dience 28-48 Hawkes and 101 Soteris. BNST was similar to the control and rehydrated animals, however the intensity of staining was diminished within PVN and SON. Fibers of the median eminence, indubitable recessus, and neuropoysis the contained Herring bodies and staining was diminished in dehydrated animals. To investigate the projections of hypothalamic Ang(1-7), True Blue was microinjected into the pituitary. 

Many neurons within the SON and PVN contained True Blue, while only a small population of these retrogradely labeled cells also contained Ang(1-7). These results indicate that Ang(1-7) in the pituitary originates. In part from neurons in the SON and PVN; however, the possibility that the density hypothalamic Ang(1-7) fibers project to other sites, such as brainstem and limbic forebrain, cannot be excluded. These findings coupled with the plasticity of Ang(1-7) in the HNS of animals with altered fluid balance suggest a contribution of this heptapeptide in fluid homeostasis. 

(NIH HL-37927, HL-6835, and George Storer Fund) 


The organization of GABAergic elements in the tuberomammillary nucleus was examined by using antibodies against gamma-aminobutyric acid (GABA) and light and electron microscopy. Most neuronal perikarya of the tuberomammillary nucleus were GABA immunoreactive (GABA-ir) and contained reaction product predominantly distributed throughout the cytoplasm and in addition, the nucleus presented intense immunoreactivity. The morphology of the GABA-ir perikarya was similar to the perikarya of the hypothalamus previously described by Hayashi et al., J. Comp. Neurol., 229:233, 1984. The GABA-ir perikarya were contacted by relatively few terminals. 

The mean bouton covering ratio of GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouton covering ratio GABA-ir dendrites in the tuberomammillary nucleus was 31%, some of the same perikarya was 6.5%, whereas the mean bouto

Using the slice explant culture method for organotypic hypothalamic cultures (Wray et al., Peptides 9:1151-1175, 1988), we have begun to study the viability of rat suprachiasmatic nuclei (SCN) in vitro. In order to determine to what extent the SCN reorganizes within the SCN placed adjacent to its appropriate phenotype, we have immunocytochemically analysed this structure using light (LM) and electron microscopic (EM) techniques. A detailed description of this nucleus and the LM showed that the SCN maintains a highly compact organization of neurons, similar to that found in vivo. Immunocytochemistry (ICC) revealed numerous neurophonic (NP) and vasoactive intestinal polypeptide (VIP)-containing neurons within the cultured SCN. Many of the putative somata of SCN cell bodies were still present after 18 days in vitro. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabeling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide and GABA-containing neurons are present in these cultures after 18 days in vitro. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days in vitro, indicates that this culture system could be a valuable in vitro model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.

341.11 ORGANIZATION OF THE HUMAN SUPRACHIASMATIC NUCLEUS. J.K. Ma1 and O.K. Ronnekleiv (SPON: European Neuroscience Association). Dept. of Neuroanatomy, University of Dusseldorf, D-4000 Dusseldorf, F.R.G.

Serial 20 um paraffin sections of five human hypothalami were processed by a modified Sternberger PAP-DAB method using antibodies (AB) to norepinephrine (NPH), vasopressin (VP), neurotensin (NT), VIP, neuropeptide Y (NPY), somatostatin (SST), myelinbasic protein (MBP), and the full-length-lysosomal enzyme (FA)-epitope. 32% of ARC neurons (N=30) had an LTS. The LTS exhibited an RMP > -50 mV (mean = 62.1 ± 1.8 mV); Rin > 85 MΩ; and a fast (Na+) spike > 50 mV with an overshoot. In voltage-clamp (N=5) was characterized as a transient outward current of up to 500 pA. Its activation/inactivation curves overlapped in the -55 to -70 mV range. Another 30% showed a slow inactivation curve with a peak near -70 mV and exhibited maximun inactivation to -70 mV. The current underlying the LTS and the cell types which exhibit this conductance were characterized in current and voltage clamp conditions onto SCN cell bodies were still present after 18 days in vitro. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabeling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide and GABA-containing neurons are present in these cultures after 18 days in vitro. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days in vitro, indicates that this culture system could be a valuable in vitro model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.

341.13 CHARACTERISTICS OF GUINEA PIG ARCULATE (ARC) NEURONS EXHIBITING A LOW THRESHOLD SPIKE (LTS) OR A TRANSIENT OUTWARD CURRENT. M.J. Kelly, M.D. Loose1 and O.K. Ronnekleiv. Dept Physiology, OHSU, Portland, OR 97201.

ARC neurons have a higher incidence of LTS when hypothalamic slices are prepared from ovariectomized guinea pigs that are estrogen treated versus oil treated. In vivo. Immunocytochemistry (ICC) revealed numerous neurophonic (NP) and vasoactive intestinal polypeptide (VIP)-containing neurons within the cultured SCN. Many of the putative somata of SCN cell bodies were still present after 18 days in vitro. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabeling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide and GABA-containing neurons are present in these cultures after 18 days in vitro. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days in vitro, indicates that this culture system could be a valuable in vitro model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.

341.10 MONONUCLEAR ANTIBODIES WHICH RECOGNIZE SUBSETS OF HYPOTHALAMIC MAGNOCellular NEUROSECRETORY NEURONS IN THE RAT.


In order to determine if neurons of the magnocellular neurosecretory system (MNS) of the rat possess cell-type specific surface molecules which could play a role in development and plasticity of the MNS, we have attempted to produce monoclonal antibodies (MAbs) against dissociated neuronal MNS neurons or membrane extracts of neuronal nuclei. The current underlying the LTS and the cell types which exhibit this conductance were characterized in current and voltage clamp conditions onto SCN cell bodies were still present after 18 days in vitro. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabeling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide and GABA-containing neurons are present in these cultures after 18 days in vitro. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days in vitro, indicates that this culture system could be a valuable in vitro model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.


Inhibitory mechanisms of endogenous kappa-agonists, leumorphin (LM) and dynorphin (Dyn), were investigated by using intracellular recordings in the supraoptic (SON) neuron of the rat hypothalamic slice preparations. Both application of Dyn and Dyn elicited slight membrane hyperpolarization with decreased spontaneous firing rate. The effects were blocked by selective kappa antagonist MR2266. LM and Dyn suppressed excitatory synaptic potentials evoked by focal stimulation dorsal to the SON. On the other hand, mu- and delta-agonists, morphine and DADLE respectively, did not influence the synaptic potentials. LM and Dyn also decreased duration of action potentials which were prolonged by TEA and Cs. The prolonged Ca spikes were blocked by NiCl2. From these results, we suggest that kappa-agonists inhibit the neural activities of neurosecretory cells in the cat SON, pre- and post-synaptically.


Intracellular recordings were made from ARC neurons in brain slices prepared from ovariectomized guinea pigs that are estrogen treated versus oil treated. Cells recorded with electodes filled with bicine were identified by staining with a streptavidin/FITC complex followed by immunocytochemistry for β-Endorphin (END) or tyrosine hydroxylase (TH). Neurons recorded using bicynyl or K-citrate electrodes had similar membrane properties in vitro. The LTS was 6-20 mV in amplitude, 30-120 ms in duration, persisted for up to 18 days in vitro and had an overshoot. 32% of ARC neurons (N=30) had an LTS. The LTS exhibited an RMP > -50 mV (mean = 62.1 ± 1.8 mV); Rin > 85 MΩ; and a fast (Na+) spike > 50 mV with an overshoot. Another 30% showed a slow inactivation curve with a peak near -70 mV and exhibited maximun inactivation to -70 mV. The current underlying the LTS and the cell types which exhibit this conductance were characterized in current and voltage clamp conditions onto SCN cell bodies were still present after 18 days in vitro. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabeling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide and GABA-containing neurons are present in these cultures after 18 days in vitro. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days in vitro, indicates that this culture system could be a valuable in vitro model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.
ANTAGONISM OF FAST EXCITATORY POSTSYNAPTIC POTENTIALS IN SUPRACHIASMATIC NUCLEUS NEURONS BY EXCITATORY AMINO ACID ANTAGONISTS: A COMPARISON OF AMPA AND NMDA RECEPTORS IN LHA NEURONS

The hypothalamic paraventricular nucleus (PVN) contains both magnocellular and parvocellular neuronal populations, which may select neural circuits in response to signals from the fastigial nucleus (FN) to the lateral hypothalamic area (LHA). In male anesthetized rats, 295 extracellular and 82 intracellular neurons, 16 were checked for change in IPSP latency with FN stimulation intensity. The aim of this study was to investigate the effects of various agents on the neuronal mechanisms underlying inputs from the FN to the LHA. The data suggest that LHA neurons receive inhibitory inputs from the FN, which may contribute to hypothalamic modulation of feeding behavior.
HYPOTHALAMUS

431.21
LOCOMOTION BY THE ANESTHETIZED RAT INITIATED BY ACTIVITY OF HYPOTHALAMIC NEURONS. H.N. Stimacyn, M. Marciello*, P. Brastow*, L.G. Clemens. Neurophysiology, Wesleyan University, Middletown, CT 06457.

Two experiments determined if locomotion in the Nembutal-anesthetized rat produced by electrical stimulation of the lateral hypothalamic (LH) was mediated by fibers of passage descending from the preoptic basal forebrain and by directly or indirectly activated neurons in the LH. Six rats received large unilateral lesions of the preoptic area and later were subjected to mapping of the hypothalamus for electrically-evoked locomotion at currents of 25 and 50 µA. Ipsilateral to the lesion, fewer LH locomotor sites positive at 25 µA were found but 4 of 6 rats had some 25-µA sites that had ipsilateral positive sites at 50 µA. In the hypothalamus of 8 intact rats injections of glutamate (50 mM, 50 nl) were made in 66 sites. Injections in the LH at the level of the hypothalamic nucleus produced short-latency (420 s) episodes of hindlimb locomotion. Descending fibers of passage contribute to, but are not essential to, the elicitation of locomotion by electrical stimulation of the LH.

431.23

The paraventricular nucleus (PVN) of the hypothalamus consisted of several subnuclei, some of which project to the neurohypophysis, others to brainstem and cervical-thoracic regions of the spinal cord. Lumbal segments L5-L6 of the spinal cord contain the sexually dimorphic motor nuclei, the spinal nucleus of the bulbocavernousus (SNB) and dorsolateral nucleus (DLN), which innervate perineal muscles (the bulbocavernousus and the ischiocavernosus, respectively). These motoneurons and their afferent input are androgen-dependent. This study examined projections of the PVN to the L5-L6 spinal cord. WGA-HRP (0.5-0.8µl) was injected into the region of L5-L6 aimed at the SNB and DLN and their dendritic extents, in intact male, castrated male or female rats. Following a 4 day survival time animals were perfused and HRP was visualized using the TMB method of Niswender (1976).

WGA-HRP labelled cell bodies were found in the parvocellular subnuclei of PVN, as well as regions of the lateral hypothalamus (LH) and the dorsal hypothalamic area (DA). These results demonstrate that the PVN projects to lumbal levels of the spinal cord that are sexually dimorphic and androgen-dependent. This suggests that the LH may modulate the activity of these motoneurons.

431.25
EFFECT OF OVARIAN CONDITION ON SEXUALLY DIMORPHIC BRAIN AREAS IN A WHITTAIL LIZARD SPECIES. J. Wade* and D. Crews*. Biology, University of Texas, Austin, TX 78712.

Both the anterior hypothalamic-preoptic area (AH-POA) and the ventromedial hypothalamus (VMH) are sexually dimorphic in the whiptail lizard, *Stenocercus miliaris*. These areas are thought to be essential to the elicitation of locomotion by electrical stimulation of the LH, which is significantly larger in females (D. Crews, J. Wade and W. Wilczynski, 1989). This work supported by NIH grant 1R01 HD41358 and by NSF EPSCoR funds. The authors acknowledge the support of the University of Texas/UT McAllen Marine Science Institute.

431.22
EVIDENCE FOR A FUNCTIONAL AND ANATOMICAL RELATIONSHIP BETWEEN THE LATERAL SEPTUM AND THE HYPOTHALAMUS IN THE CONTROL OF FLANK MARKING IN THE MOLE-RAT. C.E. Perris, L. Gold* and M. Potegal. (SPO48.8 B Gagliardi) and Departments of Psychology and Zoology, and the Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

Golden hamsters communicate by flank marking, a behavior that is controlled by vasopressin sensitive neurons in the anterior hypothalamus (AH). The magnocellular neurosecretory system is thought to be the source of neurotransmitter for the initiation of flank marking. The anatomical and functional connections between the lateral septum and the vasopressin (VP) receptor population were examined by: 1) tracing afferent and efferent connections following injection of HRP into the lateral septum, and 2) recording odor-induced flank marking prior to and following ibotenate lesions in the lateral septum. The greatest number of retrogradely labeled perikarya were found in the lateral hypothalamus, ventral to the fornix. Anterogradely labeled nerve terminals were primary. These perikarya were localized perinuclear to the VP neurons of the medial aspect of the SON. The SON, PVN and VMH were devoid of HRP-reactive nerve endings and perikarya. The injection of ibotenate into the lateral septum significantly reduced odor-induced flank marking as compared to control injections of 0.9% NaCl. The VP neurons of the medial SON receive excitatory input from the lateral septum to facilitate flank marking behavior. (NIH Grant NM223577)

431.24

The sexually dimorphic area (SDA) of the gerbil hypothalamus (hyp) accumulates gonadal steroids and is implicated in the hormonal control of male sexual behavior and scent marking. To identify pathways for these behaviors, SDA afferents were traced in both sexes by injecting Phaseolus vulgaris leucoagglutinin (PHAL)-labeled fibers. SDA afferents were verified by injectingputative terminal fields with PHAL, retrograde tracer, or both. The ventrolateral (vl) septum, medial bed nucleus (n) of the stria terminals, medial amygdala (MeA), substantia innominata, anterior periventricular n (APvN), ventromedial n (vLMH) and ventral premammillary n receive more mDA than SD. The opposite is true for the lateral hyp and retrorubral vl. Both mDA and SD project to the posterior hyp, lateral and supramammillary n, paramedial thalamic n, reunions, central gray and most raphe n. The vLMH and posteriordorsal n were most heavily labeled when the PHA-la injection overlapped the SDA. Anterogradely labeled cells were found in the SDA and parasubstantia nigra. Some elements showed sex differences in pattern, number and distribution.

431.26

Many sexually dimorphic characteristics of primates have neural bases. In rats, a similar picture exists in the hypothalamic nuclei which control reproductive function, and is associated with an estrogen-sensitive sexual dimorphism in the neural number and laminar distribution of the arcuate nuclei (NA, IPA). In vervet monkeys, they raise the possibility that other estrogen-sensitive characteristics of the hypothalamus may modulate the sexual dimorphism in the hypothalamic neural membranes. These areas are comprised of several subnuclei, some of which project to the neurohypophysis, others to brainstem and cervical-thoracic regions of the spinal cord. Lumbal segments L5-L6 of the spinal cord contain the sexually dimorphic motor nuclei, the spinal nucleus of the bulbocavernosus (SNB) and dorsolateral nucleus (DLN), which innervate perineal muscles (the bulbocavernosus and the ischiocavernosus, respectively). These motoneurons and their afferent input are androgen-dependent. This study examined projections of the PVN to the L5-L6 spinal cord. WGA-HRP (0.5-0.8µl) was injected into the region of L5-L6 aimed at the SNB and DLN and their dendritic extents, in intact male, castrated male or female rats. Following a 4 day survival time animals were perfused and HRP was visualized using the TMB method of Niswender (1976).

WGA-HRP labelled cell bodies were found in the parvocellular subnuclei of PVN, as well as regions of the lateral hypothalamus (LH) and the dorsal hypothalamic area (DA). These results demonstrate that the PVN projects to lumbal levels of the spinal cord that are sexually dimorphic and androgen-dependent. This suggests that the LH may modulate the activity of these motoneurons.

431.27
VERTEBRATE BRAIN ARE AS IN WHITTAIL LIZARDS AND ZEBRAS. J. Wade* and D. Crews*. Biology, University of Texas, Austin, TX 78712.

431.28
EFFECT OF OVARIAN CONDITION ON SEXUALLY DIMORPHIC BRAIN AREAS IN A WHITTAIL LIZARD SPECIES. J. Wade* and D. Crews*. Biology, University of Texas, Austin, TX 78712.

431.29
EVIDENCE FOR A FUNCTIONAL AND ANATOMICAL RELATIONSHIP BETWEEN THE LATERAL SEPTUM AND THE HYPOTHALAMUS IN THE CONTROL OF FLANK MARKING IN THE MOLE-RAT. C.E. Perris, L. Gold* and M. Potegal. (SPO48.8 B Gagliardi) and Departments of Psychology and Zoology, and the Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

Golden hamsters communicate by flank marking, a behavior that is controlled by vasopressin sensitive neurons in the anterior hypothalamus (AH). The magnocellular neurosecretory system is thought to be the source of neurotransmitter for the initiation of flank marking. The anatomical and functional connections between the lateral septum and the vasopressin (VP) receptor population were examined by: 1) tracing afferent and efferent connections following injection of HRP into the lateral septum, and 2) recording odor-induced flank marking prior to and following ibotenate lesions in the lateral septum. The greatest number of retrogradely labeled perikarya were found in the lateral hypothalamus, ventral to the fornix. Anterogradely labeled nerve terminals were primary. These perikarya were localized perinuclear to the VP neurons of the medial aspect of the SON. The SON, PVN and VMH were devoid of HRP-reactive nerve endings and perikarya. The injection of ibotenate into the lateral septum significantly reduced odor-induced flank marking as compared to control injections of 0.9% NaCl. The VP neurons of the medial SON receive excitatory input from the lateral septum to facilitate flank marking behavior. (NIH Grant NM223577)
MECHANISMS OF THE INITIAL PHASE OF FEVER IN THE VSA AND MAHPOA OF RATS AND RABBITS. 

Mechanisms underlying the initiation of pyrogen-induced fever were studied at two brain heat-gain sites in rats and rabbits. Three questions were addressed. The first question was to identify brain sites at which microinjection of bacterial or endogenous pyrogens evoked a clear-cut decrease in core temperature. The second question considered whether blockade of neuronal activity at these sites would modify the initial and subsequent phases of a fever evoked by bacterial pyrogens or endogenous mediators. The third question tested the relationship of PGE, the mediation of the fever responses. E, colli, PGE2, alpha-interferon, and IL-1β elicited fever from the ventral respiratory area (VRA) and MAHPOA of rats and rabbits. The fever response consisted of an early peak within 1 hr. and a 2nd peak within 3-6 hrs. The PGE2 antagonist, SC19220, or atrobe blocked fever from IL-1β in the MAHPOA. Blockade of the initial phase of the fever suggests PGE and Ach may mediate the initial events. The data demonstrate two brain sites, the VSA and the MAHPOA, in two species which are similar in their response to hyperthermic agonists, also responded to bacterial pyrogens and leucotrienes. Specific receptor antagonists at the MAHPOA site block the initial phase of a fever following microinjection of IL-1β. (Supported in part by USAPDFR-87-0297 and by NIH-R526405).

INTRACELLULAR ANALYSIS OF INHERENT AND SYNAPTIC ACTIVITY IN HYPOTHALAMIC THERMOSENSITIVE NEURONS
M.C. Curras and J.A. Boulant. Physiology Dept., Ohio State University, Columbus, OH 43210.

To understand neuronal thermosensitivity, intracellular activity was recorded in rostral tissue slices of rat hypothalamus. Neurons had mean resting membrane potentials of ~55 mV, and 30 neurons were classified according to thermosensitivity. Even though synaptic activity is present, warm sensitive neurons display inherent thermosensitivity due to a temperature dependent pacemaker potential. Neuronal cold sensitivity, however, is primarily attributed to temperature dependent changes in excitatory and inhibitory synaptic input. Cold sensitive neurons also show increased input resistance during cooling, which could enhance the effectiveness of the excitatory synaptic input. Some temperature insensitive neurons can display a “functional plasticity” in which transient periods of warm and cold sensitivity occur. This supports a previous study suggesting that temperature insensitive neurons possess a voltage dependent (thermosensitive) pacemaker current that is usually suppressed by an electrogenic Na-K pump. (Supported by NIH and the Bremer Foundation.)


Both [3H]GBR 12935 and [3H]cocaine label elements of the dopamine transporter in striatal tissue. We have directly compared the binding of drugs to sites labeled with either [3H]cocaine or [3H]GBR 12935 in caudate-putamen membranes of monkeys (M. fascicularis). Assays were performed at 4°C in Tris-HCl (50 mM) buffer containing NaCl (120 mM). The sites labeled with [3H]GBR and [3H]cocaine were 5-20 times higher than their IC50 values at [3H]GBR 12935 binding sites were 5-20 times higher than their IC50 values at [3H]cocaine receptors. Conversely, structurally unrelated monoamine uptake inhibitors including Lu 19-005, GBR 12909, and mazindol did not displace [3H]GBR 12935 fully, and their IC50 values were comparable at both sites. The results show that [3H]GBR 12935 and [3H]cocaine label related, though probably not identical, elements of the dopamine transporter. Supported by DA00499, DA00088, RR00168.

DRUGS OF ABUSE: DOPAMINE MECHANISMS


Three questions were addressed. (1) fevers evoked by pyrogens are age dependent. Experiments were conducted in both young and old rabbits to characterize the thermal responses to central injections of cytokines acting as endogenous pyrogens (PGE2, NE, and 5-HT) which could enhance the effectiveness of the thermoregulatory responses to bacterial pyrogens and leucotrienes. Specific receptor antagonists at the MAHPOA site block the initial phase of a fever (Supported in part by NIH #NS26045 to W.D.R.)


The ability of the male New Zealand white rabbit to develop fever in response to central administration of pyrogens is age dependent. Experiments were conducted in both young and old rabbits to characterize the thermal responses to central injections of cytokines acting as endogenous pyrogens (PGE2, NE, and 5-HT) which could enhance the effectiveness of the thermoregulatory responses to bacterial pyrogens and leucotrienes. Specific receptor antagonists at the MAHPOA site block the initial phase of a fever (Supported in part by NIH #NS26045 to W.D.R.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
PERSISTENT CHANGES IN BRAIN CATECHOLAMINE AND MONOAMINE LEVELS AFTER REPEATED COCAINE INJECTION TO THE RAT NEOSTRIATUM. J. Peris and R. Dawson. Dept. Pharmacodynamics, Wayne State University School of Medicine, Detroit, MI 48202.

Euphoria and reinforcement that occur with cocaine have been attributed to dopamine-mediated neurotransmission. The present study examined electrophysiological manifestations and dopaminergic mechanisms of systemically administered cocaine in the rat neostriatum. Cocaine was found to modify neuronal firing rate over a dose range of 0.1-10.0 mg/kg, while administration of drug vehicle did not result in any significant change of neuronal discharge. Of thirty-two (32) rats tested at 0.25 mg/kg cocaine, 53% were facilitated while 47% were inhibited. At higher doses of cocaine, a larger proportion of units exhibited depression after drug exposure. For example, 68% had reduced activity and 32% enhanced discharge after 0.5 mg/kg. Similar changes in neuronal activity were produced by acute infusion of L-DOPA (0.01-10.0 mg/kg) followed pretreatment with carbidopa (10.1 mg/kg). In control experiments, carbidopa itself did not modify neuronal activity.

Cocaine effects were altered by pretreatment with dopamine antagonists. Experiments with the selective DA-1 antagonist SCH 23904 reversed, blocked, or attenuated the effect of cocaine in a dose-related manner. These data indicate that the changes in neuronal activity after acute cocaine exposure may be mediated by selective dopaminergic mechanisms.

INDUCTION OF C-FOS BY DIRECT AND INDIRECT DOPAMINE AGONISTS S.T. Young, L.J. Porrino, and M.J. ladarola. (SPON: G. Dienel). LCM, NIMH and HAMH; CBND, NINDS; and NAB, NIND, Bethesda MD 20892.

The proto-oncogene C-Fos is a nuclear protein which is thought to act as a genetic transactivator regulating gene expression. Reports of alterations in c-Fos expression following dopaminergic agonist treatment prompted us to assess whether c-fos induction was a general event following dopaminergic stimulation. Fos expression was examined immunohistochemically in three sections of rat forebrain. Rats treated with the indirect dopamine agonist, cocaine (30 mg/kg, IP), showed a significant increase in Fos expression in both the number of cells and intensity of nuclear immunoreactivity in the caudate/putamen, substantia nigra, and ventromedial mesencephalon using reverse phase HPLC with electrochemical detection. Concentrations of 5-HT were significantly enhanced discharge after 0.5 mg/kg. Similar changes in neuronal activity were produced by acute infusion of L-DOPA (0.01-10.0 mg/kg) followed pretreatment with carbidopa (10.1 mg/kg). In control experiments, carbidopa itself did not modify neuronal activity. Cocaine effects were altered by pretreatment with dopamine antagonists. Experiments with the selective DA-1 antagonist SCH 23904 reversed, blocked, or attenuated the effect of cocaine in a dose-related manner. These data indicate that the changes in neuronal activity after acute cocaine exposure may be mediated by selective dopaminergic mechanisms.


Repeated exposure to high doses of methamphetamine (MA) greatly depletes cerebral dopamine (DA) and serotonin (5-HT), but does not produce gross deficits in spontaneous behavior. We hypothesized that sparing of function may occur, at least in part, because the remaining DA neurons may not be required to achieve basal extracellular (ECF) concentrations of DA. To test this idea the concentration of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) was measured in extracellular fluid (ECF) by microdialysis in freely moving rats. The animals were pretreated one week earlier with either saline or MA (15 mg/kg, i.p. x 6). MA pretreatment produced a large decrease (50-90%) in the basal ECF concentration of DOPAC, HVA and 5-HIAA, presumably reflecting the loss of DA and 5-HT terminals in striatum. In contrast, the basal ECF concentration of DA was not changed significantly by MA pretreatment. Furthermore, both saline and MA-pretreated rats showed a large increase in motor activity and the ECF concentration of DA in response to a challenge injection of 1.5 mg/kg of d-amphetamine. It is suggested that residual DA neurons can compensate for the depletion of striatal DA produced by highly neurotoxic doses of MA. Whether residual 5-HT neurons have a similar compensatory capacity is currently under investigation.


Using the quantitative 14C-[U-14C]-deoxyglucose autoradiographic method (2-DG), we compared the acute and chronic effects of methamphetamine (MA) administration on local cerebral glucose utilization (LCGU) in freely-moving rats. Four groups of animals received 0.0 (saline), 0.5, 1.0 and 2.0 mg/kg i.p. daily for 8 days and locomotor activity and changes in LCGU were measured after the first, second, and eighth injections. After a seven day withdrawal period from cocaine, rats were tested with either saline or cocaine and then seven days later were sacrificed for brain dissection. Levels of DA, NE, 5-HT, DOPAC, HVA, and 5-HIAA were measured in nucleus accumbens, striatum, frontal cortex, olfactory tubercle and ventromedial mesencephalon using reverse phase HPLC with electrochemical detection. Concentrations of 5-HT were significantly decreased in olfactory tubercle of cocaine-treated animals (0.64±0.05 vs. 0.50±0.05). There were no differences in catecholamine or monoamine levels due to cocaine treatment in any other brain region tested. These data suggest that some of the effects of repeated cocaine administration on behavior may be mediated by serotoninergic rather than catecholaminergic mechanisms.
THE EFFECTS OF SCH 23390 ON REWARDING BRAIN STIMULATION: EVIDENCE FOR PARTIAL D1 MEDIATION OF THE THRESHOLD LOWERING EFFECTS OF Mdma. M. Bird and S. Kornetsky. Boston University School of Medicine, Boston, MA 02115.

Previous studies from our laboratory have provided pharmacologic evidence that the increased sensitivity to rewarding brain stimulation caused by abuse substances in mediated primarily by dopaminergic processes. Using this model of drug-induced euphoria, we have recently shown that the reward threshold lowering caused by racemic 3,4-methylenedioxyamphetamine (MDMA) is blocked by D2 but not by 5-HT2 receptor antagonist (AAb. Soc. Neurosci. 13:1323, 1987). To further examine the role of D2 receptor subtypes in this reward system we studied the effect of D1 blockade on MDMA's enhancement of rewarding brain stimulation using the selectivity of D2 antagonist SCH 23390. Four male albino F344 rats were implanted with bipolar electrodes into the median preoptic area and the effective effects of SCH 23390 alone and in combination with a maximally reinforcing dose of MDMA were determined. In each animal reward thresholds were systematically varied by SCH 23390 and MDMA's threshold lowered. This suggests that both D1 and D2 receptors are necessary but not sufficient substrates for MDMA's pharmacologic activation of central reward pathways.


Recent evidence suggests that cocaine binds to the dopamine transporter at both low and high affinity sites. In addition, previous studies have shown that cocaine binding in Tris buffer is sodium dependent at the cocaine binding site. Since dopamine terminals in the striatum (STR) arise from the nigro-striatal neurons while the dopamine terminals in the nucleus accumbens (NA) arise from mesolimbic dopamine neurons, other laboratories suggested that cocaine can interact differently at these two areas. This is of interest since the NA has been implicated as one of the sites of cocaine's reinforcing properties, while the STR has not.

Intraplantar self-stimulation (ICSS) has long been considered a model for the hedonic effects of drugs of abuse. However, direct comparisons between ICSS and drug self-administration has seldom been possible since the characteristics of responding for 150-500 ms trains of 0.1 mA at 100 Hz are very different from those observed during self-administration of a drug with complicated absorption, distribution, and excretion kinetics. We have trained male rats to self-administer long trains of brain stimulation which are frequency-modulated to mimic the pharmacokinetic characteristics of stimulant drugs. There are striking differences between this novel form of ICSS and drug self-administration. Rats often "load up" at the beginning of a session. Increasing the "dose" administered or increasing the half-life of stimulation produces corresponding decreases in the rate of ICSS.

Furthermore, low doses of the dopamine antagonists SCH23390, pimozide, and alpha-flupenthixol, increase the "dose" of stimulation the animal self-administers. Thus, differences observed between conventional ICSS and drug self-administration behaviors may be due to the parameters of brain-stimulation. This novel ICSS paradigm may be a useful model for studying the role of pharmacokinetics in drug self-administration.

The mature structure of fetal hippocampal transplants associated with behavioral recovery (Woodruff et al., Brain Res. 433.2, 130-143, 1988) differs from that of the normal hippocampus. To begin to understand the maturity of the transplants and the reasons for the differences in behavior we studied the growth of E16 hippocampus transplants in adult rats. The volume of the transplants was estimated immediately or 30 days posttransplantation. The volume of the transplants increased significantly during the first 10 days posttransplantation. The volume of the transplants was greater than that of the normal hippocampus at 30 days. The volume of the transplants was similar to that of the normal hippocampus at 100 days. The volume of the transplants was 9.3 times that of the normal hippocampus at 30 days. The volume of the transplants was 9.3 times that of the normal hippocampus at 100 days.

433.4 EFFECTS OF SCIATIC NERVE TRANSPLANTS ON MEDIAL SEPTAL CHOLINERGIC CELLS AFTER FORNIX/FIMBRIA LESION. D.J. Miesnermich and P. Kromer, Dept. of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

Trophic and trophic effects of peripheral nerve transplants in the CNS were examined using the septo-hippocampal pathway in immature rats. Following a bilateral fornix/fimbria lesion, a segment of sciatic nerve was inserted on both sides of the brain between the septum and dorsal hippocampus. After either 2 or 8 weeks post-transplantation, 6 animals in each experimental and control (lesion only) group were perfused. Brains were sectioned at 30um and stained for cholera toxin subunit B and the number of ChAT+ cells in the medial septum was counted. The number of ChAT+ cells in the medial septum was 15% lower than that of the control group. The number of ChAT+ cells in the medial septum was 15% lower than that of the control group.

Two weeks post-transplantation there was a statistically significant increase (p<0.05) in the number of ChAT+ cells in the septum of animals receiving the transplant vs animals with lesions only (Mean ± SEM: 256 ± 28 vs 208 ± 28 cells). The number of ChAT+ cells in the septum was 15% lower than that of the control group. ChAT+ fibers were present within the nerve grafts. Our results suggest that over time sciatic nerve transplants lose their ability to maintain viable septal cholinergic neurons even though these transplants contain cholinergic axons. Further studies will examine the mechanisms of this response in detail.

Supported by NIA grant #06648.

433.5 IN VITRO STUDIES OF LC-HIPPOCAMPAL DOUBLE BRAIN GRANTS GROWN IN OCULO: AN IN VITRO ELECTROCHEMICAL STUDY. M.-T. psychiatry, University of Colorado Health Sci. Ctr., KC1 were employed to investigate the connectivity of the normal LC-hippocampal projection. In the present study, IN VITRO STUDIES OF LC-HIPPOCAMPUS DOUBLE BRAIN GRAFTS PLACED INTO ASPIRATION LESION SITES IN ADULT RATS. D.J. Paul, R.H. Reisened, and W. L. Woodruff, Dept. of Anatomy, Quillen-Dishner Col. of Med., East Tennessee State Univ., Johnson City, TN 37614.

The mature structure of fetal hippocampal transplants associated with behavioral recovery (Woodruff et al., Brain Res. 433.2, 130-143, 1988) differs from that of the normal hippocampus. To begin to understand the maturity of the transplants and the reasons for the differences in behavior we studied the growth of E16 hippocampus transplants in adult rats. The volume of the transplants was estimated immediately or 30 days posttransplantation. The volume of the transplants increased significantly during the first 10 days posttransplantation. The volume of the transplants was greater than that of the normal hippocampus at 30 days. The volume of the transplants was 9.3 times that of the normal hippocampus at 100 days. The volume of the transplants was 9.3 times that of the normal hippocampus at 100 days.


Cerebral cortex grafts significantly reduced performance associated with early fascia dentata damage may be attenuated through the use of neural grafting in mature subjects. An assessment of graft-induced behavioral benefits depends on (1) the time after transplantation that particular behavioral tests are conducted, and (2) the source and final location of the grafted neural tissue.

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433.7


The learning impairment in rats with a hippocampal lesion induced by single oral administration of trimethylamine hydroxide (TMT, 9mg/kg) were tested and compared with those in TMT-treated rats with fetal septal grafts. TMT-treatment induced a permanent increase in open-field activity 3 days after the treatment and an increased number of errors as detected by Biel water maze test which appeared as early as 4 hr and reached the steady level 5 days after the administration. Rats were treated with TMT followed a week later by implant of septal tissue taken from 14 day fetuses and digested by trypsin into bilateral 3 regions of the hippocampus. Spatial learning was evaluated by Morris water maze test 3 weeks postoperatively. The swimming time to find a submerged platform over 10 daily trials was recorded for 2 days. TMT-treated rats with sham operation exhibited a longer time spent in the water as compared with control rats and TMT-treated rats receiving septal grafts showed performance relative to TMT-rats, though still inferior to controls.

433.8


The studies presented here examined the effects of transplanted fetal cholinergic neurons on the behavioral and neurochemical alterations induced by AFA64A. This cholinotoxicosis produces long-lasting decreases in high affinity choline uptake (HACU) and the choline acetyltransferase (ChAT) activity in the hippocampus (HPC) together with persistent cognitive impairments.

Speculatively rats were trained on a single eight arm radial maze (RAM) task. Following training, rats received bilateral injections of AFA64A (3 nmol/al/cv). Ten days later, suspensions of dissociated septal cholinergic neurons (E16) or the gangliotoxin produces long-lasting decreases in high affinity choline uptake levels (HACU) and the choline acetyltransferase (ChAT) activity in the hippocampus (HPC) together with persistent cognitive impairments.

Following training, rats received bilateral injections of AFA64A (3 nmol/al/cv). Ten days later, suspensions of dissociated septal cholinergic neurons (E16) or the gangliotoxin (12.7+0.9 vs. 10.5+1.1, p>0.05). In contrast, animals receiving transplants (AF/VEH) were impaired on this task. No evidence of recovery of AFA64A produced decreases in hippocampal HACU levels (31%) and ChAT activity (58%) that were observed in the AF/VEH group. These data suggest that the transplantation of fetal septal cholinergic neurons following AFA64A promotes functional recovery in a task-dependent manner.

433.9

FETAL HIPPOCAMPAL TRANSPLANTS AMELIORATE SPATIAL MEMORY DEFICITS IN MONGOLIAN GERBILS WITH ISCHEMIA-INDUCED HIPPOCAMPAL LESIONS. S. M. Onifer and W. C. Low. Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 46223.

Cerebral ischemia produces a selective loss of CA1 pyramidal neurons in the dorsal hippocampal gyrus resulting in spatial memory deficits. The purpose of this study was to determine the effects of fetal hippocampal cells transplanted into the hippocampal formation on the restoration of spatial memory function. Transient cerebral ischemia was produced by intraperitoneal injection of 10 minutes. Ischemic and non-ischemic gerbils were trained in a Morris water maze to swim a platform visible in a training test.

The number of platform crossings (mean ± S.E.M.) in the training quadrants was determined for the ischemic and non-ischemic groups. Ischemic gerbils with sham transplants showed no improvement in spatial orienting even after transplantation (4.8 ± 6.5, p>0.05). Similarly, no improvement was observed in sham-operated gerbils (1.0 ± 1.8, p>0.05). In contrast, ischemic gerbils with hippocampal transplants exhibited a significant improvement in spatial orienting, with 18.6±10.5 crossings (p<0.05).

Basal degeneration of the host's CA1 pyramidal neurons was observed in both the ischemic group and with sham transplants. In animals with grafts, transplanted cells, morphologically similar to CA1 pyramidal neurons, were found in the CA1 field. Frequently, they were in aggregates in stratum pyramidale, overlying CA1 stratum radiatum, and also individually scattered throughout the CA1 field. Clusters of transplanted cells were also found in the stratum radiatum, subfields of stratum lacunosum-moleculare, and subfields of stratum procerum. These clusters were also found in contact with the hippocampal vasculature via penetrating vessels through the stratum radiatum. Host-derived AChE-positive fibers innervated the grafts through these axillary penetrations and the cortex. Data suggest that fetal hippocampal cells, morphologically similar to CA1 pyramidal neurons, will survive when transplanted into the CA1 field of the hippocampus of the Mongolian gerbil and are innervated by host-derived AChE-positive fibers. These grafted cells may form a new memory that results from cerebral ischemia. (Supported by AHA, Indiana Affiliate, Inc.)

433.10


Thy-1.1 to Thy-1.2 mouse congenic transplants were used to show that embryonic entorhinal area projects specifically to the appropriate entorhinal afferent territory of the outer two-thirds of the stratum molecular of the adult host dentate gyrus. This study demonstrates that the terminal field is deafferented by the removal of the entorhinal afferents. Deafferentation in turn may in turn ameliorate deficits in spatial memory that result from entorhinal afferent territory.

433.11

SELECTIVE INNERRVATION OF CA3 HIPPOCAMPAL TRANSPLANTS BY ADULT HOST DENTATE GRANULE CELL AXONS. F. M. Field*, P. J. Seeley*, M. Frotscher**, G. Rainaam (SPON Brain Research Association), Lee Research Centre, Lab. of Neurobiology, National Institute for Medical Research, London NW7 1AA, UK and Dept of Anatomy, University of Frankfurt, FRG.

We have studied formation of characteristic mossy fibre synapses between granule cell axons of adult PVG rat hosts and embryonic transplants from syngeneic rat hippocampus. Late embryonic hippocampal transplants were subdissected into its protocorticoarchitectonic fields and the quality of this subdissection monitored from the cytology of neurons and their synaptic connections. This is a prospective birthdate analysis using tritiated thymidine, staining with an antibody (py) that is selective for large pyramidal neurones of CA3, and EM analysis of identified Golgi stained CA3 pyramids and mossy fibres.

Transplanted large pyramidal neurons receive mossy fibre synapses only when the transplant makes direct contact with the host mossy fibre system and these connections are selective for CA3 compared with CA1 pyramids.

433.12

CONNECTIONS OF THY 1.2 LABELED CROSS-SPECIES TRANSPLANTS IN THE DENTATE GYRUS USING EM. B. P. Vietiev, J. Wells, and R. J. McKeon, (SPON: J. Held) Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05401.

Neural cell suspensions from the septal/basal forebrain region of Thy-1.2-positive mouse embryos were transplanted into the hippocampus of Thy-1.2 rat hosts. A Thy-1 antibody was found on the external membranes and certain internal membranes of the transplanted neurons. Within the transplant, labeled presynaptic profiles contacted labeled postsynaptic profiles. Many unlabeled presynaptic profiles contacted labeled postsynaptic profiles. Most were asymmetric synapses on dendrites of a variety of diameters. In the host, close to the transplant, many labeled fibers growing out of the transplant were often fasciculated into large groups. Each fascicle was surrounded by an unlabeled astrocytic process but there were also unlabeled processes within the fascicles. Further away from the transplant few fascicles were seen but labeled individual fibers were seen coursing through the host neuropil. There was not a consistent orientation between the labeled distal fibers and astrocytes, but a few labeled presynaptic profiles also were seen contacting unlabeled postsynaptic profiles of the host. Supported by FHS 23286.
GROWTH OF CHOLINERGIC SEPTAL-BASAL FOREBRAIN TISSUE FOLLOWING CROSS-SPECIES TRANSPLANTATION. B.J. McKeon, B.P. Vietti*, and J. Wells. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Cell suspensions of septal-basal forebrain tissue from embryonic rodent hosts were transplanted to the denate gyrus of cholinergically denervated Thy 1.1-positive rat hosts. Antibodies to Thy 1.2 and ChAT were used to compare the distribution of the donor tissue and ChAT-positive cells and processes following transplantation. Both Thy 1.2-positive and ChAT-positive cells and processes were similarly distributed within the host denate gyrus. ChAT-positive staining was most evident within the hilus and intact molecular layer. The ChAT-positive cells and processes were determined to be of donor origin since they were not present in sections of normal or lesion control animals. Thus, ChAT immunocytochemistry corroborates the selective growth of xenogeneic Thy 1.2-positive processes emanating from these cells abutting, but not crossing, the graft/host border. In contrast, numerous grafted magnocellular, NGF receptor-immunoreactive and Thy 1.2-positive cells and processes were organized in clusters in a manner similar to that observed in normal rat hosts. Antibodies to Thy 1.2 and ChAT were used to histochemically for AChE and immunocytochemically for choline acetyltransferase (ChAT). The AChE increase occurred independently of the shrinkage. Supported by PHS 23266.

FETAL MONKEY BASAL FOREBRAIN NEURONS GRAFTED INTO NUCLEUS BASALIS LESIONED CEUSB MONKEYS. J.H. Kordower*, M.S. Paslaski, and D.M. Cash. Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med., Chicago 111. 60637, USA.

Grafts containing cholinergic neurons derived from the embryonic rodent basal forebrain have been shown to survive transplantation and ameliorate some of the memory deficits which result from aging processes or lesions of either the septohippocampal pathway or basal nucleus. To date, no studies have examined the extent to which such grafts survive within the primate CNS. Four young adult (1.5-3 kg) male Cebus apella monkeys received unilateral ischemic acid lesion of the nucleus basalis. One week after the lesion, 2.5 or 5 ml volumes at a concentration of 10 mg/ml. Seven to ten days later, monkeys received cortical and amygdaloid implants of basal forebrain neurons obtained from E90 (n=2) or E110 (n=2) Cebus apella fetuses. All monkeys were sacrificed 10 weeks later. Nissl staining, nerve growth factor (NGF) receptor immunocytochemistry, and acetylcholinesterase (AChE) and diophorase (NADPH) histochemistry revealed complete remyelination of the deafferented neocortical and hippocampal neurites. ChAT and NADPH-diaphorase positive cells and processes were evident within the hilus and intact molecular layer. ChAT-positive cells and processes were determined to be of donor origin since they were not present in sections of normal or lesion control animals. Thus, ChAT immunocytochemistry corroborates the selective growth of xenogeneic Thy 1.2-positive processes emanating from these cells abutting, but not crossing, the graft/host interface. In some instances, healthy appearing Nissl stained grafts failed to contain cholinergic neurons. Numerous NADPH-containing neurons with morphological characteristics similar to those seen within the Cebus basal forebrain were observed in these transplants. These data indicate that both cholinergic and noncholinergic fetal monkey basal forebrain neurons survive well following grafting into the primate CNS. (Support: NS25653 and The American Health Assistance Foundation [JHk]).


Rats exhibit behavioral, electrophysiological, and anatomical changes as a function of aging. Fetal grafts of basal forebrain into the hippocampus and/or cortex have been shown to ameliorate some of these age-related changes. In this study we implanted 3ul cell suspensions grafts of the basal forebrain region of fetal rats (E14-15) bilaterally into the anterior hippocampus and/or entorhinal cortex of Fischer 344 rats. These aged rats had been behaviorally characterized on the Morris water maze and exhibited an impairment on this task. Upon retesting on this task following implantation of fetal grafts, the aged rats that had received grafts had significantly improved their performance to the level of aged rats that did initially exhibit an impairment on the water maze task. The implanted aged rats that did not receive grafts did not show an improvement.

AGED rats also exhibit pathological EEG patterns as reflected by frequent long-duration high voltage neocortical spindles that did not correlate with their performances on the water maze. Graft survival was demonstrated by acetylcholinesterase (AChE) staining. Immunohistochemical staining for choline acetyltransferase (ChAT) and NGF receptor (NGF) revealed the presence of ChAT-positive and NGF-positive cells in the grafts. These results suggest that grafts of fetal cells to the nucleus basalis region survive well in the aged rat, and may be able to reverse some age-related functional deficits.


The extent to which repeated administration produces tolerance to the nicotine-induced increases in dopamine transmission in the nucleus accumbens was investigated in rats. In vivo microdialysis was used to sample extracellular concentrations of dopamine and metabolites after a nicotine challenge (0.5 mg/kg) in (1) naive, (2) acutely pretreated rats (one prior nicotine injection), and (3) chronically pretreated rats (twelve to fifteen prior daily nicotine injections, 0.35 mg/kg/injection). Nicotine increased extracellular concentrations of DA and its metabolites, and these increases were not significantly altered by either acute or chronic prior exposure to the drug. The failure to find evidence of tolerance is compatible with the hypothesis that the norepinephrine dopaminergic system is a substrate for the reinforcing properties of chronically administered nicotine.

PROFILE OF THE EXTRACELLULAR CONCENTRATION OF COCAINE AND DOPAMINE UNDER SELF-ADMINISTRATION CONDITIONS. H.O. Petitt, H. Pan* and J.B. Justice Jr.. Emory University; Dept. of Chemistry; Atlanta, Georgia 30322.

Repetitive administration of cocaine leads to a buildup of both cocaine and dopamine in the extracellular fluid of the brain. A series of experiments were undertaken to monitor cocaine and DA concentrations in the nucleus accumbens under a variety of self-administration conditions in vivo. Microdialysis coupled to high pressure liquid chromatography with ultraviolet detection or electrochemical detection was used to analyze cocaine and DA concentrations, respectively. Cocaine concentrations were measured following the intravenous administration of (1) a single infusion of 0.75 mg/kg of cocaine; (2) 0.75 mg/kg of cocaine delivered in five minute intervals; (3) 1.5 mg/kg of cocaine delivered in eight minute intervals. Concentrations of DA were quantified in animals during the intravenous self-administration of either 0.25, 0.5 or 0.75 mg/kg of cocaine (doses correspond to 0.75, 1.5 and 2.25 mg/kg). Cocaine concentrations were determined to increase and stabilize within 3 minutes. Cocaine concentrations were estimated to be approximately 20 and 30 mg/kg/inj, respectively. The extracellular concentration of DA was also observed to increase and stabilize at approximately 400% of basal levels during cocaine self-administration. Results indicate that extracellular cocaine and DA concentrations are maintained at an increased, but steady level by a repetitive cocaine administration schedule.

We have found that the locomotor activity of PCP like dose of VTA dopamine (DA) neurons is correlated positively to their potency as noncompetitive NMDA receptor antagonists. However, aside from some behavioral effects similar to PCP, there is no competitive NMDA antagonist with selective, isolated dopaminergic effects. These ACh neurons are often considered the probable substrate for PCP's psychotomimetic effects.

Extracellular recordings from single VTA A10 neurons were made in chloral hydrate anesthetized rats during i.v. injections. From these recordings, cumulative dose-response curves were established for MK-801, PCP and NPC 12626. MK-801 and NPC 12626 increased neuronal firing at 0.5 and 1 mg/kg, respectively, while NPC 12626 altered firing at 5 mg/kg. The lack of an NPC effect would not appear to be due to limited access to the CNS since ip. NPC (5 mg/kg) can produce locomotor hyperactivity and ataxia like that seen with PCP. In order to determine whether the behavioral effects, like those of PCP, were mediated via mesolimbic DA systems animals received bilateral intra-accumbens injections of 6-hydroxydopamine (4 ug/str) or vehicle 14 days before challenge injections of NPC (50 mg/kg), PCP (5 mg/kg), or 6-OHDA (1.5 mg/kg) and activity measured in photocell cages. Unlike PCP and 4-AP, NPC locomotor stimulatory effects were not blocked in 6-OHDA lesioned animals.

These results suggest that, in contrast to PCP-like psychotomimetic drugs, competitive NMDA antagonists do not stimulate A10 neuronal activity nor activate locomotor behavior via mesolimbic DA systems.


Blinius et al. (Pharmacol. 85:232, 1985) have shown that following 6-OHDA lesions of the nucleus accumbens, microinjection of D-ala-Met-enkephalinamide (DALA) produces a greater increase in behavioral activity than seen in pre-lesion baseline.

In 5 subjects receiving a DALA injection each week for 5 weeks, we report a similar increase in DALA-induced behavioral activation peaking at 623 (±169) pre-lesion baseline 2 weeks after lesion, but we also report a reduction in this DALA increase to 164.7 (±71) by 5 weeks post-lesion. Baseline behavioral activity (no DALA) also increased and decreased over this period post-lesion peaking at 327 (±19) in the 2nd-3rd week after lesion.

Current work concerns attempt to identify the mechanisms of this temporary increase and application to understanding lateral hypothalamic self-stimulation.

Supported by the Whitwell Foundation


of-abstinence (with or without BROM treatment), on local cerebral glucose utilization (1CGU) in rat brain.

The increased behavioral activity and reinforcing effects of psychotherapeutic agents are believed to be primarily mediated by dopamine (DA) transmission in the nucleus accumuments (NAc). Rats self-administer D-amphetamine (Hoebel et al., Psychopharmacology, 81:158-163, 1983), but not cocaine (Goedel & Smith, Science, 221:773-775, 1983) into the N ACC.

Therefore, we examined the locomotor response and the dopamine efflux response in the N ACC during direct infusions of amphetamine and cocaine in the N ACC. Amphetamine (0.02, 0.27, 5.4, and 100umol) or cocaine (0.11, 2.35, and 470 mm) was constantly infused through a microdialysis probe into the N ACC results in increased extracellular DA concentrations, but does not produce the expected behavioral effects.

434.8


Chronic cocaine exposure may deplete neuronal dopamine (DA) stores by inducing reuptake. Systemic administration of DA receptor agonists, such as bromocriptine (BROM), could compensate for a subsequent reduction of DA output. We investigated the effects of chronic cocaine, followed by a brief period of abstinence (with or without BROM treatment), on local cerebral glucose utilization (1CGU) in rat brain.

In the first study, adult male rats were injected daily with 10 mg/kg cocaine HCl or vehicle for 14 days, and in the second study, this was followed by a 3 day period of cocaine abstinence in which the animals were injected daily with 10 mg/kg BROM or vehicle. Animals were then trained for quantitative analysis of ICGU using [14C]-deoxyglucose. LCGU experiments began 10 min following cocaine administration (first study) or 2 hr following BROM administration (second study).

Chronic cocaine resulted in a general increase in ICGU with significant increases in rostral nucleus accumbens (NAc), medial caudate, globus pallidus and substantia nigra (SN). In contrast, chronic cocaine followed by 3 days of abstinence resulted in a general decrease in ICGU in most regions studied, including NAcc, SN and ventral tegmental area. Subsequent BROM administration restored ICGU to control levels in some of these regions. Moreover, correlation analysis of ICGU data revealed that metabolic activation of mesolimbic circuits occurs following chronic cocaine exposure. These results suggest that dramatic changes occur in dopaminergic reward regions during the period of early cocaine abstinence, and that these alterations may be influenced by dopaminergic pharmacology during this period.

The mesocorticolimbic dopaminergic system has been implicated as an important neural substrate of psychostimulant self-administration. The present experiments were undertaken to electrophysiologically record single units in the nucleus accumbens and to investigate whether stimulation of this structure evokes unit discharges in the nucleus accumbens, with a stimulating electrode in the ipsilateral fimbria, and with a catheter in the jugular vein. That recorded units were unresponsive to morphine, those unresponsive to morphine were injected with 6-OHDA, a dopamine-depleting agent, and responsive to morphine were placed in a place avoidance paradigm. The data indicate that the mesolimbic dopaminergic system mediates the expression of place aversions. Rats implanted intracerebroventricularly (ICV) with morphine pellets. The animals were then subjected to place aversion training. The results suggest that the mesolimbic dopaminergic system is involved in the expression of place aversions.

434.4 NEUROLEPTIC-LIKE EFFECTS OF INTRA-ACCUMBENS CHOLECYSTOKININ ON I.V. COCAINE SELF-ADMINISTRATION. J. D'Angelo*; C.F. Nelles and G.F. Koob, Dept. of Psychology and Psychiatry. Univ. of Toronto, Canada. M5S1A1; Div. of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic.

Increased dopamine transmission in the nucleus accumbens (Acb) is an essential mechanism underlying the rewarding properties of i.v. cocaine reward. Cholecystokinin (CCK) is present in Acb DA terminals as well as non-NA neurons associated with the mesolimbic system. The present experiment examined the possible modulatory effects of intra-Acb CCK on i.v. cocaine self-administration.

Hale Vistar rats with bilateral cannulae aimed at the Acb were trained to self-administer cocaine intravenously during daily 3 h sessions. Following stable responding each rat was tested following intra-Acb microinjections of CCK (0.03, 1 and 2 ng), which produced approximately a 75% increase in counterbalanced order. A minimum of 3 no pretreatment days separated drug tests.

The results demonstrated that intra-Acb CCK produced a dose-dependent attenuation of cocaine reward as reflected by an increase in responding for i.v. cocaine. The 1 ng CCK dose, which produced approximately a 75% increase in responding, was the most effective dose. These results demonstrate that CCK may have neuroleptic-like effects on i.v. cocaine self-administration.


Brain reward is intimately associated with dopamine in mesolimbic neuronal circuitry. We studied the effect of cocaine on pre-synaptic mechanisms in nucleus accumbens, the terminal neurons for the ventral tegmental (A10) cell bodies. Cocaine in extracellular fluid was studied using microelectrode voltammetry, which was studied by Activity Pattern Analysis (San Diego Instr., Calif.). Electrochemical (ECL) measurements of dopamine, homovanillic acid after substantia nigra administration of cocaine (20mg/kg), were coupled with behavioral locomotor activity studies in freely moving, male, inbred Sprague-Dawley rats (350-450g). We then further studied the effect of cocaine on extracellular dopamine in striatum and again simultaneously recorded locomotor activity. The results show that the dopamine signal after cocaine, increased in nucleus accumbens of freely moving rats. The increase in dopamine was slow and progressive. By comparison, dopamine in striatum showed less change after cocaine administration. Interestingly, the dopamine alterations occurred before locomotor activity changes began. These data confirm cocaine’s action in dopaminergic mesolimbic circuitry. (Spons: DOD, NGR Grant #2-S07-RRO732, PSC/CUNY Award #667234 and BRSG442424 to F.A. Broderick.)

434.14 NUCLEUS ACCUMBENS AND AMYGDALA ARE SUBSTRATES FOR THE AVENGER AMNESIS EFFECTS OF OPiate WITHDRAWAL. L. Struijks*, M. Le Moal, and G.F. Koob, I.N.S.E.R.M-U.259, Bordeaux, France, and Research Institute of Scripps Clinic, La Jolla, CA 92037.

Specific brain sites for the classic opiate abstinence syndrome of wet dog shakes, plosis and teeth chattering appear to be widely represented in the brain. Using a more general rewarding components of drug self-administration. Morphine-containing cell bodies originating in the ventral tegmental area of Tsai (VTA) are a major afferent to the NAS. While VTA-derived dopamine has been proposed to mediate reward, the role of this amine for the rewarding effects of opiates is unclear. We have investigated the effects of systemic and local infusions of morphine on spontaneous and evoked activity of NAS neurons in anesthetized rats. Systemic or iontophoretically applied morphine generally inhibit randomly encountered spontaneously active NAS cells. However, phrenetically or systemically applied morphine are uniformly ineffective in altering limbic-driven NAS activity recorded from normally ‘silent’ NAS units. On the other hand, micro-infusion of morphine (2.5 µg in 1 µl) into the VTA (VTA-M) inhibits this limbic-driven unit discharge, and subsequent administration of morphine (s.c.) can reverse this VTA-M inhibition suggesting an opposing action of opiates at a separate, non-VTA site. In fact, micro-infusion of morphine into the ventral subiculum, but not into the amygdala, reverses the inhibitory effect of VTA-M on limbic-evoked NAS activity. These data suggest that the effects of systemic morphine on NAS unit activity result from the integration of opiate-sensitive NAS afferents having potentially opposing actions on NAS activity. (Supported by DA03695, K02DA00131 and AA07456 to S.J.H.)

434.12 OPIATES INFLUENCE NUCLEUS ACCUMBENS NEURONAL ACTIVITY BY DOPAMINE AND NON-DOPAMINE MECHANISMS. S.J. Hennenken and R.L. Hakan, Department of Neuropharmacology, The Research Institute of Scripps Clinic, La Jolla, CA 92037.

The nucleus accumbens septi (NAS) of the telencephalon is thought to mediate, in part, reward and aversive components of drug self-administration. NAS neurons receive dopaminergic input from the VTA and projections from the amygdala, ventral tegmental area, nucleus accumbens, and perhaps the amygdala, are critically involved in the aversive reinforcing properties of opiates. The present study examined whether IC administration of MN produced aversive stimulus effects as measured by the formation of place aversions. Rats implanted intracerebroventricularly (ICV) or with bilateral cannulae aimed at the medial dorsal thalamus, periaqueductal gray, ventral tegmental area, amygdala or nucleus accumbens were made dependent on morphine by subcutaneous administration of unit 75 mg morphine pellets. The animals were then subjected to place aversion training by pairing of a distinct environment with a single ICV injection of MN. Results showed that at high doses (100 ng/mg) of MN produced a place aversion. However lower doses (250-500 nanograms) produced significant brain site selectivity with the region of the nucleus accumbens most sensitive. Observations of aversive stimulus effects were made using the acute period with the high dose of MN showed various signs of opiate withdrawal at most sites except the amygdala where no classic abstinence signs were observed. Recent results indicate that the amygdala is critically involved in the aversive stimulus effects of opiate withdrawal. (This work supported by NIDA grant DA04043.)

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434.15 INTRA-ACCUMBENS INJECTION OF THE GLUTAMATERGIC ANTAGONIST GDEE INHIBITS THE LOCOMOTOR-ACTivating EFFECTS OF COCAINE AND AMPHETAMINE, BUT NOT STIMULATION INDUCED BY APOMORPHINE. F. I. Callahan, S. B. Leshay and C. E. Koob, 1, Dept. Psychiatry, UCSD, La Jolla, CA 92037. 2, Scripps Clinic & Res. Fdn., La Jolla, CA 92037. 3, Dept. Neurology, Univ. Pavia, Italy.

Studies of the neurochemical substrates of the locomotor activating properties of psychostimulants have focused on brain dopamine (DA) systems, particularly the mesolimbic dopamine system and its terminal fields in the nucleus accumbens (NAC). Advances in our understanding of the effenter and afferent connections of the NAC suggest that the behavioral effects of psychostimulants might be modulated within the NAC by glutamatergic afferents originating within allocortex. We examined the effects of blockade of NAC glutamate activity on the locomotor-activating effects of cocaine (COC) and amphetamine (AMP) - drugs that stimulate locomotion by enhancing NAC DA activity - and caffeine (CAF), which stimulates locomotion independent of NAC DA.

These results suggest that the locomotor-stimulated effects of drugs that enhance NAC DA activity are modulated by NAC glutamate transmission. Glutamate projections to the NAC from allocortex may modulate the behavioral properties of NAC DA activity.

434.17 NORCOCAINE INHIBITS THE SPONTANEOUS ACTIVITY OF DORSAL RAPHE (DR) SEROTONIN NEURONS IN THE RAT. Joseph M. Parris, Kathryn A. Cunningham and Joan M. Lakoski. Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Cocaine has been demonstrated to inhibit the activity of catecholaminergic and serotonergic (5-HT) neurons in the CNS. Norcocaine is the primary oxidative metabolite of cocaine which also inhibits synthesis, tyrosine hydroxylase content, and decreased 3H-spiroperidol binding in several brain areas of the rat. Synthesis, tyrosine hydroxylase content, and decreased 3H-spiroperidol binding in several brain areas of the rat. Synthesis, tyrosine hydroxylase content, and decreased 3H-spiroperidol binding in several brain areas of the rat.

Studies of the mechanisms underlying the effects of cocaine on neurotransmission in rats following chronic cocaine self-administration show that allocortical glutamate afferences to the NAC may be involved in modulating the NAC function in drug reinforcement. Supported by NIDA DA04296.

434.19 LACK OF NEUROCHEMICAL EVIDENCE FOR NEUROTOXIC EFFECTS OF REPEATED COCAINE ADMINISTRATION IN RATS ON BRAIN MONOAMINE NEURONS. S.Y. Yeh and E.B. De Souza. (SPON: R.L. Frank & B.P. Williams). Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221.

Midbrain dopaminergic pathways and opioid receptor systems have been implicated in the rewarding effects of intracranial self-stimulation. Evidence points to the ventral tegmental area as the site of the interaction of these two systems (Yeh, Fed. Proc. 404, 1987). These results show that the endogenous glutamate activity in the NAC may be of importance for the maintenance of iv cocaine self-administration in rats. Glutamate afferents to the NAC are thought to originate mainly from the hippocampus and the amygdala, this is the first evidence suggesting allocortical modulation of the NAC function in drug reinforcement. Supported by NIDA DA04296.

434.20 COCAINE: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF EFFECTS IN AMYGDALA NUCLEI. K.A. Cunningham, P.M. Callahan and H.A. Lekan. Dept Pharmacology/Toxicology, Univ Texas Medical Branch, Galveston, Texas 77550.

The limbic system may regulate action for cocaine since this stimulant alters psychological processes mediated by this circuitry, including mood and emotion. Cocaine inhibits the uptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT), systems which intervene in limbic circuits such as the amygdala which is sensitive to cocaine-induced "kindling". Using extracellular recording techniques, we have begun to characterize the effects of cocaine on the activity of amygdala neurons of rats anesthetized with chloral hydrate (400 mg/kg). Of 20 neurons recorded to date, 14 were located in basolateral and 6 in central amygdala. The spontaneous activity of these neurons was slow and irregular with an average rate of 1.2 Hz (range: 0.1-8.5 Hz); triphasic (+/−/+) action potentials were most typical. Using extracellular recording techniques, we have begun to characterize the effects of cocaine on the activity of amygdala neurons of rats anesthetized with chloral hydrate (400 mg/kg). Of 20 neurons recorded to date, 14 were located in basolateral and 6 in central amygdala. The spontaneous activity of these neurons was slow and irregular with an average rate of 1.2 Hz (range: 0.1-8.5 Hz); triphasic (+/−/+) action potentials were most typical. Using extracellular recording techniques, we have begun to characterize the effects of cocaine on the activity of amygdala neurons of rats anesthetized with chloral hydrate (400 mg/kg). Of 20 neurons recorded to date, 14 were located in basolateral and 6 in central amygdala. The spontaneous activity of these neurons was slow and irregular with an average rate of 1.2 Hz (range: 0.1-8.5 Hz); triphasic (+/−/+) action potentials were most typical.
435.3 PROTEIN SYNTHESIS IN THE VENTROMEDIAL HYPOTHALAMUS IS MODIFIED BY SMALL PULSES OF ESTRADIOL. C.D. Condon, D.M. Lynn*, and E.J. Roy. Neural and Behavioral Sciences, Department of Psychology, University of Kentucky, Lexington, KY 40506.

The role of estrogen and the ventromedial hypothalamic nuclei (VMH) in reproductive behavior of the rat has been well documented. A hypothesis of estrogen action in the VMH is that estrogen increases synthesis of specific proteins. The purpose of this investigation was to establish if estrogen's mechanism of action in the VMH is by modulating protein synthesis and if so, what proteins are affected.

Twelve, 60 day old Long Evans female rats were ovariectomized and bilateral dorsolateral funiculus (DLF) transections were made. Animals were maintained on a cyclic sex hormone schedule and were trained to acquire a radial 8-arm maze task. Following acquisition of criterion performance, rats were injected with 2 pulses of 4 µg/kg 17β-estradiol, 12 hrs apart (6 controls received 0.3 ml 0.1% saline). At the time of the second pulse, each rat was given 4 intracerebroventricular (icv) injections of 2µl of 2% aluminum wire, 0.01% tryptamine, 100nM serotonin and 0.9% saline. Rats were then killed by decapitation. The VMH was isolated, homogenized and run on 2-dimensional SDS-PAGE electrophoresis. The resulting film was analyzed by an Olympus Cue-2 computerized image analyzer. The results confirm that estrogen modulates VMH activity by altering protein synthesis. Interestingly, many of the low molecular weight proteins affected by estrogen are significantly reduced by the hormone.


The effects of midbrain electrical (0.2 ms, 10-100 Hz, 150 pulses, 50-500 µA) and chemical (0.01-0.1 M Na-L-glutamate, 20µl) stimulation on sexual behavior of male and female monkeys were examined. Six male and three ovariectomized, estrogen-treated female rhesus monkeys were used in accordance with the NIH Guide (1985). Six of the six male monkeys, touching, sometimes showing lordosis. The asymmetric transections spare periventricular regions of the VMH and adjacent tegmentum. One response was elicited with a mean latency of 7 s. The other, with longer latency (>15 s), was a long-lasting repetitive response. The present study suggests that different areas of the midbrain are involved in the control of sexual behavior of male and female monkeys.

435.6 CENTRAL GRAY CONNECTIONS WITH THE VENTROMEDIAL HYPOTHALAMUS ARE ESSENTIAL FOR LORDOSIS IN FEMALE RATS. Ann C. Hennen, Larv CAMAK and David A. Edwards. Department of Psychology, Emory University, Atlanta, Georgia 30322.

The central gray is believed to play an important role in the control of sexual receptivity in female rats, but published reports relating central gray destruction to lordosis in female rats describe only modest deficits in sex tests with males. We show that appropriately placed central gray lesions and knife-cuts virtually eliminate lordosis, and we also provide compelling evidence that central gray connections with the ventromedial hypothalamus are essential for female sexual behavior. Details follow.

Electrocorticographic recordings of the midbrain-pontine central gray (CG) and its lateral surround eliminated lordosis in ovariectomized females injected with estradiol and progesterone. In a second study, sagittal knife-cuts which bracket the central gray at the level of the rostral pons also eliminated lordosis. The central gray is necessary for the expression and maintenance of lordosis behavior, in contrast with the ventromedial hypothalamic nuclei (VMH) which is necessary for the expression of lordosis behavior.

To establish an assay relevant to reproductive behavior which also tests for the female’s disposition to receive the mating site. Females were ovariectomized and all compared: 4 days of E, 14 days of E, and 4 days of E plus the door leading from the first chamber was partially closed, thus requiring the female to deliberately leave the mating site. Females were ovariectomized and all received 1 mg estradiol (E) in oil. A 4-day test period was given with the females being in the E + P treated females were significantly different from the other two groups. They showed more lordoses per 20 min test (p = .006) and spent a smaller percent of the time out of the first chamber (p = .04). Thus, behavior paced by the female (cf. Femy, Behavioral Neurosciencse, 1986) yielded a strong progestrone effect. These baseline data will be used to evaluate drugs relevant to somaestosensation.


Both systemic and intraventricular administrations of the cholinergic muscarinic antagonist, scopolamine, have been shown previously to inhibit naturally occurring sexual behavior in intact, cycling female rats (Harvard & Dehanch, Physiol Behav 55:145, 1989). The present study attempted to examine cholinergic facilitation of sexual behavior in intact, cycling female rats. In the first experiment, intraventricular infusion of the acetylecholineesterase inhibitor, eserine (10µg bilaterally), did not facilitate lordotic responses when infused 15 min after administration when infused during Diestrus I, Mid-diestrus, or Diestrus II. In a second experiment, however, intraventricular infusion of eserine did facilitate lordotic responding 15 min (p<0.001) and 1 hr (p<0.0006) after administration when infused during Early Proestrus and Proestrus. Cycling was determined by daily monitoring of sexual behavior and vaginal cytology. As previously reported with the administration of other cholinergic agents, infusion of eserine did not significantly interrupt cyclicity patterns. Because estrogen levels are highest during Proestrus and cholinergic facilitation appears to be limited to this time, it is suggested that estrogen priming of central cholinergic systems is necessary for the cholinergic regulation of sexual behavior in intact, cycling female rats.

IBOTENIC ACID OR 6-OHDA LESIONS OF THE VTA INHIBIT SEXUAL RECEPTIVITY IN HAMSTERS. C.A. Frye* and J.F. DeBold. Dept. Psychology, Tufts University, Medford, MA 02155.

Proestrone (P) implants in the VTA facilitate receptivity in estrogen-primed hamsters, whereas electrolytic lesions to the VTA inhibit receptivity. Since electrolytic lesions are nonspecific, we assessed the ability of axon-sparing lesions of the VTA to inhibit P facilitated receptivity. 5 µg ibotenic acid was infused into the rostral VTA of several hamsters. After one week, these animals were given 10 µg EB and two days later either 10 or 100 µg P. Ibotenic acid lesions of the VTA substantially inhibited receptivity to P (p<0.001) when infused into the VTA. Neither controls nor experiments responded to 10 µg P.

Because the VTA includes the A10 dopamine (DA) cell group we also examined the effects of DA selective lesions on receptivity. 7.5 µg 6-OHDA was infused into the VTA, with desethylamphetamine given so 30 minutes prior. 6-OHDA caused a partial bilateral lesion in the VTA reduced the facilitative effects of 100 µg P on the lordosis duration. After 100 µg P the controls had longer mean lordotic bouts than the 6-OHDA group. These results with axon-sparing lesions indicate that damage to fibers of passage do not explain our earlier results which suggest that the VTA is an important region in the control of sexual receptivity in hamsters.

MORPHINE INFLUENCES ON ESTRUS BEHAVIOR AND MONOAMINE RELEASE FROM THE VENTROMEDIAL HYPOTHALAMUS. J. Vathy and A.M. Eigen, Depts. Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

It is well known that morphine (M) inhibits estrogen and progesterone-dependent female sexual behavior. M has also been shown to decrease norepinephrine (NE) release in several brain regions. Our previous study employing microdialysis of the ventromedial hypothalamus (VMH) of freely moving rats demonstrated a substantial increase in NE release when females were actively engaged in mating behavior. Thus the present experiments using microdialysis to test the hypothesis that M inhibition of sexual behavior is associated with depressed NE release from the VMH. Monamine levels in dialysates from awake, behaving animals were monitored throughout the estrus cycle, with selective lesions on receptivity. 7.5 µg 6-OHDA was infused into the VTA, with desethylamphetamine given so 30 minutes prior. 6-OHDA caused a partial bilateral lesion in the VTA reduced the facilitative effects of 100 µg P on the lordosis duration. After 100 µg P the controls had longer mean lordotic bouts than the 6-OHDA group. These results with axon-sparing lesions indicate that damage to fibers of passage do not explain our earlier results which suggest that the VTA is an important region in the control of sexual receptivity in hamsters.

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ESTROGEN AND PROGESTERONE EFFECTS ON REPRODUCTIVE BEHAVIOR. G. Baimbridge, C.A. DeBold, and R.L. Moss. H. E. Hollingsworth, VA Medical Center, Dallas, TX 75235.

The role of the vromosporal organ (VNO) on the display of mating behavior was studied in ovariectomized rats using two different experimental conditions. In the first experiment, VNO-removed (n=15) or sham-operated (n=11) females were primed with 2ug estradiol benzoate (EB) a day later by followed 42 hours after progesterone (P). For six to six hours after P injection, the females were tested for reproductive behavior. The preference of the females for a sexually active male over a castrated male, proestrous females and an ovx-ized female was not affected by VNO removal. However, the VNO-removed females exhibited less preceptive behaviors and more repressive behaviors as the sham-operated contain the shams. Even though the lordosis to mount (L/M) ratio was relatively high in the VNO-removed females (mean 81.0%), it was significantly less as compared with that in the sham-operated controls (mean 96.9%) (p=0.018).

In the second experiment, VNO-removed (n=12) or sham-operated (n=13) females were injected with 2ug EB. Beginning at 48 hours after EB priming, the females were tested for sexual receptivity in every 30 min for a period of 5 hours. The enhancement of sexual receptivity following repeated mating was significantly reduced in the VNO-removed females as compared with that in the sham-operated females.

The results suggest that the VNO plays a modulatory role in sexual behavior in female rats. Supported by NIH-MH41784.
CHANGES IN OXYTOCIN RECEPTOR BINDING ARE CORRELATED WITH CYCLIC FLUCTUATIONS IN OVARIAN HORMONE LEVELS. S. Pliskin.

The present study, in vivo receptor autoradiography was used to examine the estrogen-dependent induction of OT receptor binding in brain areas implicated in sexual behavior. OT receptor binding was assessed. Animals were killed on the day in which sexual receptivity was observed (proestrus) and 24 h (estrus) or 48 h (diestrus) later (N=6/group). Animals were killed by decapitation. Blood was collected for plasma steroid assays and brains were removed and frozen on dry ice. Brief slices (25um) from the VMN were cut on a cryostat and labeled with 50pmol [3H]OVT. After 2h of in vitro binding, the slices were washed and OT receptor binding was measured using specific OT receptor antagonists as the reference standard.

Changes in OT receptor binding were correlated with fluctuations in circulating steroid levels. In agreement with other studies, high levels of estrogen and progesterone were found in proestrous animals, with intermediate levels occurring in diestrus and low levels during estrus. This study demonstrates changes in VMN OT receptors occurring during the estrous cycle and may be involved in the regulation of steroid-dependent reproductive behavior.

LONG-TERM POTENTIATION IV


Recent evidence has raised the possibility that a blockade of LTP may contribute to the effectiveness of MK-801 in inhibiting the development of kindled seizures, (Abraham et al., Brain Res., 462, 1988). Gilbert, Brain Res., 463, 1998; McNamara et al. Neuropharmacol., 27, 1988). To test this hypothesis, New Zealand rabbits, deeply anesthetized with halothane, were implanted with stimulating and recording electrodes in the entorhinal cortex (EC) and the dentate gyrus (DG). Following surgery, rabbits were injected with penicillin then were allowed 2 weeks to recover. For three days prior to kindling, the GC response to 10 PF impulses applied at each of 10 locations was determined. On the fourth day, MK-801 (0.5 mg/kg, ip) was injected and 2 hours later kindling stimulation was applied to the PP. Significant LTP of the PP-GC response occurred following kindling. The magnitude of LTP was greater at 24 hours after kindling.


Several authors have suggested that long term potentiation (LTP) may be a major component of the mechanism of kindling. If this is so, treatment that affects LTP should retard kindling similarly and vice versa. We tested this by administering APV and urethane to different groups of rats and subjecting them to LTP of the perforant path-dentate cortex, or both. Afterkindling, APV and urethane treatments were started 13th AD indicated that MK-801 completely blocked the population spike 24 hr post train stimulation. Kindling was induced by daily delivery of stimulus trains (2 s, 60 Hz, 1 pulse duration) applied at each of 10 locations. Of these groups of rats urethane markedly delayed the onset of kindling. Afterkindling, APV and urethane treatments were started following kindling. The magnitude of LTP was greater at 24 hours after kindling.

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MODULATION OF HIPPOCAMPAL PRIMED BURST POTENTIATION BY ADRENAL CORTICOSTERONE. M.C. Bennett. D.M. Diamond. M. Fleshner and o b t a in e d  fro m  120  n e u r o n s  i n  t h e  p r e l i m b i c  a r e a  o f  t h e  PFC

The magnitude of PB-LTP evoked in ADX+CORT rats which had low potential (peak latency 19.4 ± 0.54 ms) was measured off

The magnitude of PB-LTP evoked in ADX+CORT rats which had high potential (peak latency 19.4 ± 0.54 ms) was measured off

In SHAMs, the magnitude of PB-LTP was negatively correlated with

though to release the preprovasopressin (PPP) peptide vasopressin (VP, neurophysin II (NPH) and C-terminal gamma-endorphin (CTP 1-39) in the lateral septum (LS). VP and CTP 1-39, but not NPH, facilitate the glutamate/Glu-ergic transmission between the fimbria (FI) fibers and LS neurons. With the augmentation of VP (wave x 0.3 probability) evoked by stimulation of the FI fibers, we examine effects of training in shuttle-box on this transmission in the rat LS. After 10 trials of 10 trials, the acquisition and extinction sessions and the responses of the rats were recorded. The FFs recorded before and after the acquisition and extinction were compared. All the FFs classified as learners (6 or more correct responses in the last acquisition trial), showed 20-30% increase in the N-x wave of the FPs after the acquisition compared to baseline. The N wave before and after the acquisition and extinction was high average 10% higher compared controls. After extinction, the N wave increased again. The bad learners (less than 5 correct responses) showed only a 10-20% decrease in the FPs N-wave after both the acquisition and extinction. The diabetes insipidus rats of the Brattleboro strain, either good or bad learners, showed only a decrease in the N-wave of the FPs after the acquisition and extinction. Thus, the training in shuttle-box can release principles in the LS that for hours facilitate the Glu-ergic transmission between the fimbria fibers and LS neurons. These principles might be PPP-like peptides.

LONG-TERM POTENTIATION OF EARLY AND LATE EXCITATORY POSTSYNAPTIC POTENTIALS IN KITTEN VISUAL CORtical CELLS. I. Konorski and K. Konorska. Dep't of Physiology, Kyushu Prefectural Univ. of Med., Kaminokyo, Kyoto 602, Japan.

Synaptic transmission during and after conditioning stimulation (CS) of white matter (NMDA) receptors, respectively. 2) eEPSP was depressed during CS and potentiated after CS, while iEPSP was potentiated during CS and depressed after CS. 3) Long-term potentiation (LTP) of e- and iEPSPs occurred together (7/13) or separately (1/13 after eEPSP, 3/13 for iEPSP) after strong CS. 4) Weak CS produced only LTP of iEPSP in a few cells (2/8), in which the LTP was potentiated during CS. 5) NMDA antagonists did not affect LTP of eEPSP but they blocked iEPSPs. These results suggest that the two LTPs are based on different mechanisms.

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436.11

UNEXPECTED PROPERTIES OF HEBB-SYNAPSES IN RAT HIPPOCAMPAL SLICE CULTURES. T. Borbory, V. Staiser*, and A.M.H.J. Vermeulen** (SPON: V. Bratton for Biological Cybernetics, Speyererstrasse 38, 74074 Tubingen, FRG.)

In order to study the nature of synaptic enhancement in more detail we investigated whether hippocampal slice cultures (Gähwiler, B.H., J. Neurosci. Meth. 4:239, 1980) displayed plasticity. We showed that phenomena like LTP do exist in this preparation, we used a paradigm developed by Gustafson et al. (J. Neurosci. 7:774, 1987) to enhance CA1-synapses. CA1-synaptic enhancement to the Schaffer-collaterals was paired with post-synaptic depolarizations using an intracellular electrode in the cell body of the dentate granule cell (data from the dentate gyrus). Gustafson et al. showed that this enhancement is spatially restricted on the postsynaptic dendrite: if one input to the postsynaptic neuron is strengthened, the strength of a neighboring input to the same neuron is not affected. In order to assess whether this observation also holds for the presynaptic axon (i.e. whether it is also spatially restricted on the input fiber) we performed the following experiment. A stimulating electrode was placed in the afferent fibers, two closely adjacent CA1-cells (distance 20-25 mm) were impaled with intracellular electrodes, and responses to a test stimulus were recorded from both of them. We enhanced synapses by pairing 30 single extracellular stimuli to the afferent fibers with depolarizing current pulses in one cell but not in the other cell. We examined the response of both cells before, during, and after the pairing. In all experiments in which we could induce synaptic enhancement in the 'paired' cell (n=7 out of 13), we observed that the responses of both cells were enhanced, although only one of them had received paired stimulation. This result was confirmed with optical recordings using voltage-sensitive dyes: over an area of some 150 µm around the paired cell all recording sites showed significant enhancement.

These findings suggest that synaptic enhancement by the paired stimulation paradigm is not strictly local on the presynaptic axons; even local post-synaptic stimulation results in 'synaptic recruitment'. The synapses of many neighboring postsynaptic cells are enhanced.

436.13

PRECISE TEMPORAL INTERACTIONS DISTINGUISH INDUCTION OF LTP FROM LTD IN THE RAT DENTATE GYRUS. H. Hashemabadi-Gargari and W.B. Levy, Dept. of Neurosurgery, Box 425, Univ. of VA, Charlottesville, VA 22908.

Levy and Steward (83) showed that temporally staggered activation of the ipsilateral (IPS) and the converging contralateral (CNT) entorhinal cortex projections to the dentate gyrus results in either potentiation or depression of the CNT synaptic response. Depression results when the CNT activation follows IPS activation; potentiation occurs when the IPS activation precedes the CNT activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. These earlier experiments used 8 pulse (400 Hz) trains which gave a temporal resolution of 3.175 ms regarding the timing during conditioning. The present study used 3 pulse (400 Hz) trains in a similar staggered conditioning paradigm. Conditioning periods consisted of 16 of these brief trains (1 train: 20 ms). Four responses were measured: the IPS and CNT responses of both sides of the brain. The paradigm consisted of three conditioning periods with baseline response size established for 30 minutes before and after each conditioning period. In the first conditioning period the time between trains was 7.5 ms; in the second the time between trains was 2.5 ms. The last period also used 2.5 ms but reversed the IPS/CNT order (intertrain times are measured from the last pulse of the leading train to first pulse of following train). The statistically significant results with the 9 rats confirmed the ordering effect: CNT potentiation ensued when a CNT train lead a IPS train; with the reverse ordering CNT depression ensued in all cases (that is, whether or not prior potentiation or depression had been induced). The IPS responses potentiated. These results imply that the biochemical/biophysical reactions which determine whether potentiation or depression ensues from all but identical activation paradigms must account for differences of 7.5 ms or smaller. W. Holmes pointed out that there is a delay in NMDA receptor conductances which might account for this temporal sensitivity. Supported by NS15488 and NIMH RSDA K02-MH00622 to WBL.

436.14

LOCAL BLOCKADE OF INHIBITION UNMASKS A CAPABILITY FOR LTP IN THE DENTATE COMMISSURAL PATHWAY THAT IS OTHERWISE NOT EXPRESS. D. Steward, R. Tomasulo*, and W.B. Levy. Dept. of Neuroscience and Neurosurgery, Univ. of VA, Charlottesville, VA 22908

LTP can be readily elicited in a number of hippocampal pathways, but is not expressed in the dentate commissural pathway. This pathway is similar to the commissural/Schaffer collateral projection to CA1, except that it activation produces powerful inhibition that occurs nearly concurrently with the excitation. The present study evaluates whether this inhibition prevents the pathway from expressing LTP. Acute neurophysiological experiments were carried out in urethane anesthetized rats. To locally block inhibition in the dentate gyrus, a recording microiontophoresis filled with 50% bicuculline in saline was positioned in the dentate gyrus. A control saline-filled microiontophete was positioned nearby. The commissural pathway was activated by stimulating electrodes in the contralateral CA3/CA4 region. High frequency stimulation of the commissural pathway reliably elicited LTP at the bicuculline electrode, but not at the control electrode. This LTP required a threshold level of stimulation for its initiation. The high frequency stimulai induced an extracellular negativity at the bicuculline electrode that was not present at the control electrode. This negative potential was selectively blocked by ketamine and MK-801, suggesting that the negative potential reflects NMDA receptor activation.

These results suggest that LTP is not normally expressed by the dentate commissural pathway because the simultaneous inhibition clamp the post-synaptic membrane at a potential that prevents the depolarization-related relief of Mg* * blockade of the NMDA receptor. Removal of inhibition would then relieve this blockade of NMDA receptor activation, enabling LTP. Supported by NSF Grant #NS5818760 to OS and RSDA MH00622 to WBL.

436.15


The induction of LTP is thought to depend on Ca* * influx through NMDA-receptor channels. Ca** influx at a synapse on a dendritic spike and the resulting change in free Ca* * concentration in the spine head were studied in a model of a hippocampal dentate granule cell as a function of input frequency and the number of co-activated afferents. These modeling studies provided a number of interesting insights. First, in order for the description of the NMDA-receptor mediated synaptic conductance used in the model to be consistent with previous experimental observations, the average number of open, unlocked NMDA-receptor channels on a single spine head at a given moment had to be very small (usually less than one). Second, no more than a four-fold change in Ca* * influx was observed by increasing input frequency or by increasing the number of co-activated synapses (to 115%). Four responses were measured: the IPS and CNT synaptic response. Depression results when CNT activation follows IPS activation; potentiation results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation. Depression results when CNT activation precedes or is just simultaneous with IPS activation.
437.1
Rats recovered from a subacute bout of thiamine deficiency and pyrithiamine treatment provided a model of degeneration that allowed the study of non-specific aspects of impaired performance of behaviors requiring representational memory and the consistent occurrence of thalamic lesions. In this study, rats were injected with the internal medullary lamina. To discover the effects of these lesions on thalamocortical projections, a group of six long-Evans rats were lesioned following the treatment recovery for 1 to 2 weeks sacrificed and brain tissue processed for Fink-Heimer and cresyl violet staining of alternate serial sections through cortex. Signs of dense axonal degeneration were observed in layers 3 and 4 in areas of frontal, parietal, and occipital cortex. In individual animals, patterns of degeneration were bilaterally symmetric. These data suggest that the apparently limited lesions of this model of amnesia affect specific thalamic inputs in widespread areas of cortex.

437.2
There has been considerable emphasis on the role of prefrontal cholinergic projections in learning and memory, but somewhat less on their attentional function. Relatively specific destruction of cholinergic cells in the nucleus basalis (NB) of the marmoset (M. nemestrina) was achieved by infusing quisqualic acid into the substantia innominata (SI, cortico-thalamic cholinergic) and the ventral pallidum (VP, nigro-thalamic cholinergic). These lesions impaired the rats' accuracy in localising visual targets in a 5-choice serial reaction time task. The deficit in choice accuracy was in contrast to an initial retention deficit, as seen previously, but normal ED shift performance, as compared to controls. These results suggest that the deficits resemble the effects of orbitofrontal and amygdaloid lesions, rather than lesions of dorsolateral prefrontal cortex.

437.3
Lesions of the SI/VP using quisqualic acid can be employed to produce more specific reductions in cortical cholinergic markers than other excitotoxins. Such lesions fail to result in the marked behavioral deficits on a range of tests, calling for a re-evaluation of the behavioral effects of excitotoxic lesions of the cortical cholinergic projection. The present study investigated the effects of quisqualate-induced lesions of the SI/VP (which produce ChAT reductions of 50-60% in frontal and parietal cortex), on an operant, discrete trial discrimination reversal paradigm which is also sensitive to ibotenate lesions of the SI/VP. A comparable deficit was demonstrated in which the SI/VP lesioned rats performed as well as controls on a brightness discrimination but were impaired when the contingencies were reversed. Lesioned rats inhibited the previously correct response normals but were slower than controls to learn the response that had been previously incorrect. These results will be discussed in relation to the role of the cholinergic projections within their various target sites, especially the frontal cortex, amygdala, and compared to those found following lesions of the SI/VP in the marmoset.

437.4
There has been considerable emphasis on the role of prefrontal cholinergic projections in learning and memory, but somewhat less on their attentional function. Relatively specific destruction of cholinergic cells in the nucleus basalis (NB) of the marmoset (M. nemestrina) was achieved by infusing quisqualic acid into the substantia innominata (SI, cortico-thalamic cholinergic) and the ventral pallidum (VP, nigro-thalamic cholinergic). These lesions impaired the rats' accuracy in localising visual targets in a 5-choice serial reaction time task. The deficit in choice accuracy was in contrast to an initial retention deficit, as seen previously, but normal ED shift performance, as compared to controls. These results suggest that the deficits resemble the effects of orbitofrontal and amygdaloid lesions, rather than lesions of dorsolateral prefrontal cortex.

437.5
Previous studies have used excitotoxins such as kainic or ibotenic acid to examine the role of local and far consequences of nucleus basalis (NB) lesions. In the present study, rats were given bilateral injections of colchicine into the NB. The animals were examined for changes in learning and memory. Unlike excitotoxins, which can produce extensive subcortical damage, colchicine produced a lesion limited to the site of injection. Histological studies of the colchicine lesioned rats demonstrated the number of choline acetyltransferase (ChAT) positive cells in the NB, and resulted in a marked loss of cortical acetylcholinesterase staining. Separate neurochemical analysis showed that choline acetyltransferase activity in the neocortex but not the hippocampus or caudate nucleus. Rats with NB lesion showed a large deficit in a passive avoidance task, and were transiently impaired during acquisition of a reference memory task in the Morris water maze. In a reversal test in the water maze, the learning deficit reappeared. These data suggest that colchicine may be useful in producing specific lesions of the NB, which primarily affects the rate of acquisition of a spatial reference memory task.

437.6
Choline chloride supplementation during development has been shown to produce lasting effects on cholinergic function and improve spatial memory that extends well into adulthood (Mock et al., Dev. Psychobiol. 21, 1988). We now report initial findings indicating that perinatal choline supplementation alters the distribution of cells in the VDB that show NGF receptor immunoreactivity in perinatally choline treated rats and their controls. NGF binding sites were examined in horizontal sections through the diagonal band at 3 levels (bregma -6.6, -7.3, and -8.1 mm) using a marker less of cortical acetylcholinesterase staining. Separate immunocytochemical analysis showed that colchicine lesioned rats had somata that were larger, rmember, and had more dense and more abundant controls and the caudal-rostral distribution of cells in the VDB was altered by perinatal choline treatment. These results suggest that perinatal choline supplementation may alter cholinergic function and improve spatial memory through enhancement of NGF/NGFR system.
ANALYSIS IN THE DIAGONAL BAND AND DENTATE GYRUS. Warren R. Meck and Christina L. Williams. Department of Psychology, Columbia University and Bard College, New York, New York 10011. Choline chloride supplementation during embryonic days 12–16 and later (during postnatal days 16–30) has been shown to produce long-lasting facilitation of spatial memory processes (Meck et al., in press). We now report that these effects are not equivalent to those that result in learning and memory deficits, are not associated with increased cholinergic cell body clustering in the horizontal limb of the diagonal band. In contrast, the choline effect that is produced during ERB 437.9 is dependent upon steroid input, and is associated with increased dendritic length and branching in the dentate gyrus. These time frame differences cannot be related to neurotransmitter, neuronal death, and synaptogenesis in the basal forebrain and hippocampus, respectively.

INCREASED WORKING MEMORY CAPACITY AS A FUNCTION OF PRE- AND/OR POSTNATAL CHOLINE ENRICHMENT: A CORRELATION STUDY AND CORRELATED GOLGI ANALYSIS IN THE DIAGONAL BAND AND DENTATE GYRUS. Warren R. Meck and Christina L. Williams. Department of Psychology, Columbia University and Bard College, New York, New York 10011. Choline chloride supplementation during embryonic days 12–16 and later (during postnatal days 16–30) has been shown to produce long-lasting facilitation of spatial memory processes (Meck et al., in press). We now report that these effects are not equivalent to those that result in learning and memory deficits, are not associated with increased cholinergic cell body clustering in the horizontal limb of the diagonal band. In contrast, the choline effect that is produced during ERB 437.9 is dependent upon steroid input, and is associated with increased dendritic length and branching in the dentate gyrus. These time frame differences cannot be related to neurotransmitter, neuronal death, and synaptogenesis in the basal forebrain and hippocampus, respectively.

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437.13

VISUAL REVERSAL-LEARNING DEFICITS AFTER THALAMIC LESIONS IN PIGEONS. L. Chaves* and W. Rodos, Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

Shimizu and Rodos (Behav. Neurosci., 1989) reported lesions of the paleostriatum augmentatum (PA) and the hyperstriatum ventrale (IMHV), both targets of the thalamofugal pathway, resulted in increased errors in a color reversal-learning task. This finding suggested that the thalamofugal pathway might play a role in visual discrimination involving stimulus-context changes.

In the present study, lesions of the OPT complex (the thalamic source of afferents to IHA and HD) were found to have no effect on reversal-learning performance. Instead, we found that damage to nucleus rotundus (the thalamic component of the tectofugal pathway) resulted in deficits that were far in excess of those that had been obtained after IHA and HD lesions. We suggest that the reversal-learning deficits after wulst lesions are not due to the wulst's connections with the thalamofugal pathway, but rather to its connector with the tectofugal pathway.

437.14


Three distinct nuclei of the chick forebrain—the intermediate medial hypothalamus ventralis (IMHV), lateral preoptic area (LPO), and paleostriatum augmentatum (PA)—show metabolic, morphological, and neurophysiological changes following training of day-old chicks on a passive avoidance task. We compared effects of lesions in the IMHV or the LPO on the ability of chicks to learn and retain the avoidance task.

Bilateral lesions placed 24 hr before training in the IMHV produced an impairment in avoidance responding tested three hours after training. Pre-training unilateral lesions in the left but not the right IMHV resulted in a similar impairment. Bilateral IMHV ablations, given either one or six hours post-training, did not impair retention. In contrast, pre-training unilateral lesions of the LPO were not amnestic. Bilateral lesions of the LPO given one hour post-training produced significant amnesia. We are currently examining the effects of post-training unilateral LPO lesions.

These results are consistent with other studies that have examined the effects of bilateral IMHV lesions on acquisition and extend these findings by demonstrating lateralization of acquisition involving the left IMHV. The results also suggest that the IMHV is not necessary post-training to retain the memory for the avoidance training. The results of the LPO lesions indicate that the LPO is not necessary for acquisition of the avoidance response, but may maintain the memory trace following training. Hypotheses to account for these results and indications of future research will be discussed.

Supported by NIMH 1F32 MH09626 and SERC Grant GRE 57413.

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Symposium. NEUROPEPTIDE REGULATION OF REPRODUCTION. Paul E. Micewych, UCLA (Chairperson); R.B. Simler, Salt Inst.; C. Mabry, Memorial Univ.; S.P. Kalra, Univ. of Florida; S. Qieta, Oregon Reg. Primate Ctr.

The regulation of reproduction by the nervous system involves the integration of endocrine and sensory cues. In the CNS, this integration has been attributed to a sexually dimorphic, gonadotropin-sensitive circuit that includes specific loci in the amygdala, hypothalamus and brainstem. This symposium will examine both the role of gonadal steroids in modulating the function of this circuit and also the regulation of steroidogenesis by peptide inputs to the ovary. Paul Micewych will correlate the accumulation of gonadal steroids in SP and CCK-immunoreactive cells with the distribution of SP- and CCK-receptors of these populations. Robert Simler will describe the selective gonadal steroid regulation of progesterone-CCK mRNA as well as the regulation of androgen and estrogen receptor mRNA. Charles Mabry will present evidence that gonadal steroids maintain SP- and CCK-like inputs to loci in the circuit and that SP in this reproducively relevant circuit acts to facilitate reproductive behaviors in male and female rats. Sandra Qieda will discuss the regulation of gonadotropin secretion, the effects of gonadal steroids on NPY secretion, and the interaction with the endogenous opioid system. Sergio Ojeda will complete the presentations by elucidating functional roles for the VIPergic and sympathetic innervation of the ovary and their effects on the development of this organ.

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Symposium. INHIBITORY INFLUENCES ON GROWTH CONES AND CELLS. M.E. Schwab, Univ. of Zurich (Chairperson); J. Raper, Univ. of Pennsylvania; F. Bonhoeffer, Max Planck Inst.; D. Raper, Univ. of Florida; K. Ehrismann*, Friedrich Miescher Inst., Switzerland; S.B. Kater, Colorado State Univ.

Substrates favoring neurite growth as well as outgrowth promoting and chemotactic soluble factors are critically involved in nervous system development. Recently, however, several lines of evidence point to the existence of antagonistic, inhibitory mechanisms. Non-permissive substrate properties restricting neurite growth or cell migration can be associated with cell surface or ECM components. In addition, specific neurotransmitters can arrest growth cone mobility. Inhibitory components were found in membranes of neurons of specific subsets of neurons (J. Raper), in a regionalized pattern in the embryonic chicken tectum (F. Bonhoeffer), and on oligodendrocytes and CNS myelin (M.E. Schwab). For tenascin, a non-permissive substrate found in the ECM of developing PNS and CNS, the molecular analysis reveals a domain structure and leads to models for the mechanism of action (R. Chiquet). The drastic inhibitory effect of neurotransmitters on growth cones seems to involve calcium-mediated mechanisms (S.B. Kater).

Thus, neurite growth and tract formation in the developing NS may be crucially influenced by inhibitory mechanisms at various stages of development.

441.1


This analysis also provides a formal mathematical method for back-mapping VI onto the retina. In previous reports, we find that OD stripes may back-map to horizontal "slices" in the visual field. Computation of binocular visual functions may take advantage of this horizontal projection of OD stripes. Conversely, the arrangement of the OD stripes could be configured postnatally by circuits that promote stripe formation along the direction of greatest disparity, that is, the horizontal.

441.2

NONINVASIVE COMPUTATIONAL CARTOGRAPHY OF HUMAN VISUAL CORTEX BASED ON MAGNETIC RESONANCE IMAGING (MRI) AND POSITION EMISSION TOMOGRAPHY (PET). G.J. Carman and B.M. Moral, Division of Biology, Caltech, Pasadena, CA 91125.

The development of algorithms for the production of unfolded maps of the cerebral cortex (1) has made it possible to map extensive areas of the human cortex for the first time. Here we report the application of these techniques to data obtained from MRI and PET scans of the brain of a single human subject so as to yield a mapping of cerebral blood flow (CBF) as a function of location on an unfolded map of the cortex. Contours delineating the surface of cortex were digitized from MRI scans and used to compute a three-dimensional reconstruction of the cortical surface through application of differential geometry. Since the cortical surface is intrinsically two-dimensional, it can be "unfolded" by computing a mapping of the reconstruction onto the plane. An approximately conformal mapping, in which measures of length and angle are preserved with an absolute minimum of distortion, can be obtained through application of the optimization technique known as "stretched annealing" (1). Once this mapping is obtained, PET data in register with the MRI data can be projected onto the unfolded cortex so as to yield a map of differential CBF as a function of cortical location arising from controlled visual stimulation.


Previous studies have shown the lateral geniculate nucleus (LGN) of the prosimian primate, Galago, to be composed of three pairs of layers characterized by magnocellular (Y-like), parvocellular (X-like) and koniocellular (W-like) arbors. In the present study, we injected FIAU into the W-like LGN layers, reconstructed the arbors that terminated in striate cortex (area 17) then compared the location of these arbors to the patterns of cytochrome oxidase (CO) on alternating stained sections. The results show that all W-like arbors terminate within individual somatotopic columns in layer III of area 17. Many of these arbors bifurcate in layers V or VI and produce a collateral that arborizes in layer I. No axon possessed collaterals that terminated in more than one blob or in any other cortical layer. The Y-like arbors are about the same size (0.027 ± 0.013 mm^2) as X-like arbors that terminate in layer I and Y-like arbors (0.08 ± 0.013 mm^2) that terminate in layer IVc. While W-like arbors have fewer boutons than Y-like arbors, they have about the same number of boutons as X-like arbors. This suggests that the W-like pathway could provide significant input to the CO-blobs in these primates. (Supported by EO1778 to VAC & M90754 to EAL).

A DOUBLE-LABEL 2-DEOXYGLUCOSE (2-DG) STUDY. C. Redies and M. Diksic*. Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4.

The visual cortical organization in the orientational domain was studied in six NO2O2-anesthetized ferrets using a simplified version of the quintuple double-label 2-DG technique (Redies and Diksic, Neurosci., 1989). 5 mCi of [1H2-D2]- and 25 mCi of [14C2]-DG were used as tracers. Stabilized lasted 60 and 30 min, respectively. Cortical activation patterns were separated in each animal using a subtraction procedure that corrects for uptake of the first tracer during the second stimulation (Redies et al., Neurosci. 22: 501, 1987) and for cross-contamination between the isotypes.

Stimulation with two gratings of orthogonal orientation elicited activation patterns that are partially complementary and show little overlap between orientation columns. Areas not activated by either stimulation suggest a mosaic-like pattern with intermediate orientations represented in relatively large spaces between the orthogonals. With orientations differing by 45 degrees, a complex pattern of partial overlap is observed. The orientational changes show frequent alterations in their magnitude and direction. No evidence of any modularity was found. The maps obtained in this study are strikingly similar to computer-generated maps simulating development (Redies and Diksic, PNAS 83:8779, 1986). Supported by MRC of Canada (MA-10233).
DIRECT DEMONSTRATION OF THE RELATIONSHIP BETWEEN INTRINSIC AND EXTRINSIC CORTICO-CORTICAL CONNECTIONS USING DOUBLE-LABEL TRACT TRACING. B. Malach Center for Neurosciences, Weizmann Inst. of Science, Rehovot, Israel 76 100.

The relationship between intrinsic (within a cortical area) and extrinsic (between cortical areas) horizontal connections was studied using double label tracetracing approach. Single rows of clusters of neurons in the fluorescent tracer Dii were placed in cat area 18. The resulting label in area 17 consisted of a set of banded patches which were revealed in the whole fixed brain preparation. Under visual guidance, a crystal of the tracer Dii was inserted into one of the BB-labelled patches. Following 2 months of incubation, the Dii produced intense fiber labelling, which, in tangential sections, appeared to radiate from the injection site, preserving several tongues and patches. Comparing the BB labelled extrinsic patches and the Dii labelled intrinsic connections revealed clear instances of overlap but no cases of interdigitation in the material studied. The applicability of the double label approach for revealing the relationship between intrinsic and extrinsic connections in different cortical areas and different mammalian species will be assessed.

Supported by grants BSF 85-00258. Inst. Psychobiol.

CORTICAL CONNECTIONS OF INFERIOR TEMPORAL CORTEX IN SQUIRREL MONKEYS. R. E. Waller, J. F. Hood* and C. E. Steele. Dep. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

All primates appear to have a large region of inferior temporal (IT) cortex that is visual in function. We determined the connections of caudal IT cortex in squirrel monkeys (Saimiri sciureus) by making injections of neuroanatomical tracers in different locations in caudal IT, such as the ventral temporal cortex, ventral IT, lateral V II, and locations in the temporal sulcus and medial to the inferior temporal sulcus. These intra-IT connections suggest the existence of subdivisions within IT cortex in squirrel monkeys. Supported by NIH grants R29 EY07147 to R.W. and T32 EY07033 to G.S.


In monkeys, visual information reaches inferior temporal (IT) cortex via projections from an area of cortex called V4 in macaque monkeys and the dorsolateral area (DL) in owl monkeys. V4 extends from lateral to ventral cortex, while DL is restricted to the dorsolateral surface. The present study examined which of these organizational schemes exist for cortex in the location of caudal DL in squirrel monkeys. Supported by NIH grants R29 EY07147 to R.W. and T32 EY07033 to G.S.

INDUCTION OF A PLACENTAL-LIKE ALKALINE PHOSPHATASE AND REDUCTION IN PROTEIN TYROSINE PHOSPHORYLATION CONCURRENT WITH BUTYRATE-INDUCED NEURONAL TRANSFORMATION OF THE TE671 GLIAL LINE. Maria B. Marrero and Ronald J. Lukas (SPOR, H.N. Siegel). Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

We have previously reported that the TE671 human medulloblastoma cell line exposed to 'differentiating agent' sodium butyrate undergoes morphological transformation to a state resembling that of mature neurons in a time- and concentration-dependent manner. These cellular morphological changes involving neurite extension and a 2-3-fold increase in alkaline phosphatase activity, and an induction of a placenta-like alkaline phosphatase isoenzyme as shown by Western (immunoblot) analyses all are apparent two-three days of treatment with 2 mM sodium butyrate. Western immunoblots probed with a monoclonal antibody against phosphotyrosine indicate that butyrate treatment induces a striking decrease in the phosphotyrosine content of a number of proteins, but that these effects occur within one hour of butyrate treatment. All of these effects are maintained for the duration of butyrate exposure. The results suggest that several mechanisms involving cellular protein phosphorylation/de-phosphorylation might be involved in butyrate-induced neuronal transformation of the TE671 cell line.

CHARACTERIZATION AND PARTIAL PURIFICATION OF NEURON SPECIFIC GLYCOPROTEINS FROM DROSOPHILA MELANOGASTER. Munho-Maine, V.* and Salvaterra, P. (SPON: K. Ikeda). Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Antibodies directed against the plant glycoprotein horseradish peroxidase (HRP) recognize an epitope expressed by all neurons at different stages of Drosophila nervous system development. The isolation and characterization of the specific neuronal antigen(s) bearing the anti-HRP epitope(s) could be a potentially valuable tool for studying neuronal development. We have used Western blots of Drosophila proteins, stained with anti-HRP antibodies, to characterize and partially purify the glycoproteins from the Drosophila nerve cord.

Detergent extracts from larval stages show a single immunoreactive polypeptide with a molecular size of 52 kD. Immunostaining is no longer observed when the extracts are passed through an antibody specific to the carbohydrate portion of the glycoprotein, indicating that the epitope recognized by the antibodies is a carbohydrate (i.e. a glycoprotein). This glycoprotein appears to be heterogeneous with respect to its carbohydrate composition. Purification of the antigen from second instar larvae, using detergent extraction, anion exchange chromatography and preparative gel electrophoresis results in a 10% pure sample of antigen which is being used to generate monoclonal antibodies to the protein core.

In contrast to larval stages, a homogenate from adult fly heads shows 2 immunostained polypeptides of 43 and 70 kD. Both bands are at least partially aqueous soluble. The anti-HRP staining of both bands is sensitive to periodate oxidation.
DIFFERENTIAL EXPRESSION OF CATECHOLAMINERGIC AND ANGIOTENSIN II RECEPTORS IN ASTROCYTIC GLIAL CULTURES FROM NEONATAL AND 21-DAY-OLD RAT BRAINS.

M.K. Raizada.* L. Horkv* and S.P. Baker. Departments of Physiology and Pharmacology & Therapeutics, University of Florida, College of Medicine, Gainesville, Florida. 32610

Catecholaminergic and peptidergic receptors have been implicated to play a role in the development and differentiation of the CNS. However, attempts to investigate the mechanism of their involvement has been hampered, in part, by the absence of homogenous cell populations in the CNS. We have established astrocytic glial cells (AGC) in primary culture from the brains of neonatal and 21-day-old rats in order to address this issue. By using the cell culture system one may observe that the fetal rat brain from E12 to birth, while α subunit (Rlα, RII/S, Cα) mRNA levels are abundant in both primary neuronal and glial precursor cells. After about E12, cAMP may act only via the α subunit (Rlα, RII/S) in neuronal cultures, but are abundant in primary neuronal cultures. Thus, prior to about E12, cAMP may act only via the α subunit in neurons. (Supported by NIH GM 35903 (to RAM), NS 46229 (to REF) and Univ. of Iowa Diabetes and Endocrinology Research Center Grant DK 25295.)
ONTOMETRY OF BRAIN GLUCOSE METABOLIC RESPONSE TO COCAINE: A QUALITATIVE AUTORADIOGRAPHIC STUDY. D.L. Dow-Eddwards and S.A. Freed Lab of Cerebral Metabolism, SUNY-Health Science Center, Brooklyn, NY, 11203.

We have published long term metabolic changes in rats exposed to cocaine during the pregnancy (Dow-Eddwards et al., 1988). However, the acute effects of cocaine on brain glucose metabolism during ontogeny have not been demonstrated.

At 7, 14, or 21 days of age, nontreated pups were injected with either saline or 25 mg/kg cocaine-HCl, and held in an incubator at 35°C. Ten minutes later, 1.1 µCi 2-deoxyglucose was injected and the animals were decapitated 45 minutes later. Brains were removed, frozen and processed for autoradiography. Optical density patterns of cortical and subcortical structures were determined using a computerized image analysis system.

At 7 days of age, none of the brain regions appeared to be stimulated, while the thalamus appeared to be metabolically depressed. At 14 days of age, patterns of increased activity appeared in several cortical regions as well as the zona incerta and subthalamic nucleus. At 21 days of age, brain glucose metabolic response to cocaine was similar to that seen in the adult rat (Porro et al., 1988). The results are discussed in relation to the ontogeny of the behavioral responses to cocaine (Spear & Brick, 1979).

Supported by ADAMHA Grant DA0418.
443.3
THE DISTRIBUTION OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY (CCKLI) IN ISOCORTEX IN NORMALS AND ALZHEIMER'S DISEASE. Mark A. Cole and N.W. Kowall (SPON: S.K. Kostky). Neurology Service, Massachusetts General Hospital, Boston MA 02114.

Our previous findings have indicated that there is an antibody in the CSF from some AD patients which recognizes cholinergic neuronal populations in the rat central nervous system (CNS). In order to further investigate the specificity of this CSF antibody neuronal cultures of cholinergic cells were employed. Cholinergic cultures were prepared from dissociated cells from the inferior olivary nucleus of rats and neurons were identified based on their response to cholinergic agonists. These neurons were labeled with a monoclonal antibody specific for the muscarinic receptor antagonist, [H]-pirenzepine, and a nonspecific binding antibody was added to the wash. We have shown that the antibody recognizes cholinergic neurons in culture as well as in the CNS. Addition of a-NGFR (1/200) to cholinergic cells destroyed the neuronal population within 5 days. Addition of CCKLI to AD CSF and to AD patients' serum did not destroy cholinergic cells. However when a-NGFR antisera was added to neurons incubated with patient CSF samples only those cultures incubated with AD CSF and with a-NGFR antibody lost the ability to bind to cholinergic neurons. These data provide further support to our hypothesis that the presence of IgG in the CSF of AD patients may participate in the pathogenesis of this neurodegenerative disease.

443.4
INHIBITION OF HISTAMINE METABOLISM BY THA (9-AMINO-1,2,3,4-TETRAHYDROACRIDINE). F. Cummings, P.B. Reiner and S.B. Vincent. Kinmen Laboratory of Pharmacology and Neurology, University of Miami School of Medicine, Miami, Florida, USA.

THA is a compound that has been reported to have protective effects on nerve cells in vitro and in vivo. In the present study, we have investigated the effect of THA on the metabolism of histamine in the brains of aged mice. We have found that THA is able to inhibit the metabolism of histamine, as measured by the uptake of [14C]histamine into brain slices. This inhibition is dose-dependent and is reversible by the addition of histamine. These results suggest that THA may have some potential as a therapeutic agent for the treatment of neurological disorders characterized by histamine deficiency, such as Alzheimer's disease.

443.5
LOSS OF GUANINE NUCLEOTIDE-SENSITIVE HIGH AFFINITY AGONIST BINDING TO M1 MUSCARINIC RECEPTORS IN ALZHEIMER'S DISEASE. D.D. Flynn and D.C. Mash, Departments of Pharmacology and Neurology, University of Miami School of Medicine, Miami, FL 33101.

Diminished cholinergic neurotransmission is thought to play a major role in the development of Alzheimer's disease neuropathology. The failure of cholinergic replacement therapies to alleviate specific features of the symptomatology may reflect a diminished responsiveness of postsynaptic muscarinic receptors (putative M1 receptor subtype) to direct and indirect-acting cholinergic agonists, since the number of these receptor sites appears to be unchanged throughout the course of the disease. A recent report on M1 receptor-G protein coupling has been suggested in a preliminary study which used one point carbachol-[H]-pirenzepine assays on cerebral cortex homogenates from AD subjects (Peeters et al., Neurosci. Lett. 91:257, 1987). Using full carbachol-[H]-pirenzepine displacement assays, we demonstrate that M1 receptors show a 100-fold difference in their high (Kd) and low (Kl) affinity agonist states. High affinity binding is converted to low affinity by the addition of the nonhydrolyzable GTP analog, GppNHp (0.2 mM). Our results further demonstrate that the proportion of M1 receptors in the high affinity agonist state is significantly diminished in AD, with a concomitant loss in sensitivity of the Kd state of the receptor to GppNHp. We are currently attempting to correlate variations in the M1 receptor system with age of onset, disease duration and severity. Supported by NS 19065 and NS 25785.

443.6

PET studies of glucose metabolism in patients with Alzheimer's Disease (AD) and in patients with Pick's Disease (PK) often reveal reduced metabolism in parietal and frontal cortices. We have recently observed with SPECT similar regional patterns of uptake of the muscarinic receptor antagonist [123I]iodoQNB. To evaluate the comparability of the data from these techniques, 10 subjects (4 AD, 2 AD, 2 AD) were examined for uptake of [123I]iodoQNB. On the glucose PET scans, reduced metabolism was observed in parietal and frontal cortex. With SPECT, the distribution of muscarinic receptor binding was reduced in parietal cortex in AD patients and in frontal cortex in PK patients. However, one of the PK patients had basal ganglia hypometabolism despite good ligand uptake above normal levels. These results suggest that PET and SPECT may be complementary techniques for the study of dementia.

443.7
ANTIBODIES IN THE CEREBRO-SPINAL FLUID (CSF) OF ALZHEIMER'S DISEASE PATIENTS: INVESTIGATION USING CHOLINERGIC NEURONAL CULTURES. C. Dahlstrom, A. Wigander, K. Lundmark* and T.N. Chase, Department of Psychiatry, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Cholinergic neuronal cultures were prepared from dissociated cells from some AD patients which recognizes cholinergic neuronal populations. The cholinergic neurons were identified based on their response to cholinergic agonists. These neurons were labeled with a monoclonal antibody specific for the muscarinic receptor antagonist, [H]-pirenzepine, and a nonspecific binding antibody was added to the wash. We have shown that the antibody recognizes cholinergic neurons in culture as well as in the CNS. Addition of a-NGFR (1/200) to cholinergic cells destroyed the neuronal population within 5 days. Addition of CCKLI to AD CSF and to AD patients' serum did not destroy cholinergic neurons. However when a-NGFR antisera was added to neurons incubated with patient CSF samples only those cultures incubated with AD CSF and with a-NGFR antibody lost the ability to bind to cholinergic neurons. These data provide further support to our hypothesis that the presence of IgG in the CSF of AD patients may participate in the pathogenesis of this neurodegenerative disease.
443.9 SPECIFIC ALTERATIONS IN CALCIUM BINDING PROTEIN GENE EXPRESSION IN NEURODEGENERATIVE DISEASES. A. M. Lacapone* and S. S. Chariakos. Biochemistry and Molecular Biology, University of New Jersey Medical School, Newark, NJ 07103.

We have utilized a specific cDNA for the vitamin-D-dependent calcium binding protein, calbindin-D_28k, in order to examine changes in its gene expression in the amyloid and human brain as well as in 3 neurodegenerative diseases (Parkinson's, Huntington's, Alzheimer's). Changes in calbindin-D_28k gene expression were characterized by Northern and RT-PCR analysis (all blots were reprobed with β-actin and calmodulin cDNAs to determine specificity). Using gross brain regions in the aging rat, significant decreases in calbindin-D_28k mRNA were seen in the cerebellum (4-fold), subcortical area (9-fold) but not in the cerebral cortex. Using more specific brain areas in the aging human brain, significant decreases in calbindin-D_28k mRNA levels were seen in the cerebral (6-fold), corpus striatum (5-fold), and nucleus basalis (7-fold) but not in the neocortex, hippocampus, locus coeruleus, amygdala, or nucleus raphe dorsalis. Comparison of diseased human brain tissue with age/match controls yielded significant, specific decreases in calbindin-D_28k mRNA for substantia nigra (Parkinson's, 5-fold), corpus striatum (Huntington's, 8-fold), and nucleus basalis (Alzheimer's, 8-fold) but not in the amygdala, neocortex, or locus coeruleus. Since the calbindin-containing neurons in these areas are particularly affected in each of the disease processes, it is suggested that a decrease in calbindin may lead to a failure of intraneuronal calcium homeostasis and calcium-mediated irreversible cytotoxic events during the pathological processes.


444.4 BENEFICIAL EFFECTS OF PLATELET ACTIVATING FACTOR (PAF) ANTAGONIST IN EXPERIMENTAL NEUROINJURY IN RATS. K.U. Prehmoa, P.J. Lindseb, J.M. Hallenbeckc and G.S. Feuerstein. SBP-BSO-Cox) Dept. of Neurology, USH-2N2, Bethesda, MD 20814

The pathomechanisms of secondary brain damage after ischemic or traumatic brain injury are far from understood. Since PAF has been recently discussed to be a potential mediator in neuroinjury, we studied the effects of the novel platelet-activating factor antagonist BN-52021 on lesion volume, focal and highly discrete cortical neuroinjury in rats, in a model currently being determined. Funded in part by ADRA.

444.10 ALTERED cAMP-DEPENDENT PROTEIN PHOSPHORYLATIONS IN POSTMORTEM BRAIN TISSUES AFFLICTED BY ALZHEIMER'S DISEASE. W. Willigoc, K.M. Parks*, L. Bremer, K. D. Per*, Y. Farkasauth, and Department of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029

We have examined protein phosphorylation patterns in human postmortem tissues by the back phosphorylation assay, in which phosphorylating kinase activities are measured as the in vitro incorporation of phosphate into various endogenous phosphoproteins. The cAMP dependent phosphorylons were measured as the amount of phosphate incorporated into two specific and well characterized substrates, Synapsin I and pyruvate dehydrogenase (PDH). Although the degree of phosphorylation varied with the tissue sample, this variability was inherent in the sample and not due to the assay. Sample variability did not correlate with either age of the brain or postmortem interval suffered by the tissue. In animals that died within 24h, the degree of phosphorylation of PDH in the same tissues was measured in the same samples. In addition, no differences were detected with either substrate within the two groups. Of the many specific proteins examined similar differences inphosphate content were observed by autoradiography. These differences are not due to the absolute amount or structural identity of the substrates. Whether these phosphorylations are a result of a change in the expression or activity of cAMP dependent protein kinases or due to altered endogenous cAMP kinase activity or altered in situ state of phosphorylation of the substrate proteins is currently being determined. Funded in part by ADRA.
444.3 DYNORPHIN A ATTENUATES NMDA RECEPTOR-MEDIATED CORTICAL NEURONAL INJURY IN VITRO. D.W. Choi, K. Rose*, J.R. Selzer and J.R. Davis*, Dept. of Neurosurgery, Virginia Commonwealth University, Richmond, VA 23298. DYN, a potent NMDA receptor antagonist, prevents the NMDA neurotoxicity associated with hindlimb paralysis, nociceptive loss and spinal cord ischemia when injected into the lumbar cistern. Since NMDA receptors have been implicated in the pathophysiology of ischemic CNS injury, we examined the effects of DYN on MK801, a non-competitive NMDA receptor antagonist, on DYN-induced histopathological changes. Adult S-D rats were divided and injected intrathecally as: Group I-saline, Group II-DYN, and Group III-DYN+MK801. After neurological evaluation for 72 hours, the rats were perfused, the lumbosacral cord was removed, and serial cross-sections were analyzed using H & E Nissl, Kliver-Barrera and Bielschowsky techniques. Samples were evaluated by three blinded observers. Interrater agreement and differences between groups were statistically analyzed. Spinal tissue from I appeared normal while tissue from II exhibited lumbosacral cord necrosis. The injury was characterized by widespread ischemic cell changes, neuronal cell loss and axonal degeneration in the gray and adjacent white matter, with diffuse gliosis and cellular infiltration. Tissue from III exhibited small islands of necrotic tissue or slight ischemic changes, restricted to the gray matter. In addition, there was mild gliosis and edema. Thus, DYN improved neurological and neuropathological outcome in DYN-treated animals.

444.4 PRETREATMENT WITH MK-801 REDUCES BEHAVIORAL DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY (TBI) IN RATS. B.G. Lyeth, L.W. Jenkins, B.J. Hamm, L.L. Phillips, H. Young, G. Clifton*, and R. Haves. Division of Neurosurgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298. Several lines of evidence suggest that excitatory agonist-receptor interactions at muscarinics (Lyeth et al, Brain Research 452:299,1988) and NMDA (Hayes et al., J. Neurotrauma 5:587, 1988) sites contribute to pathophysiological consequences of TBI. The present study examined the effects of the non-competitive NMDA antagonist, MK-801 on behavioral deficits associated with fluid percussion TBI in the rat. Rats (n=10/group) were administered either saline or MK-801 (0.1, 0.3 or 1.0 mg/kg, i.p.) 15 min, and Metoatine 5 min prior to moderate fluid percussion TBI. Rats were ventilated as necessary following injury. Rectal temperature was monitored prior to and for 40 minutes after injury. Brain temperature was monitored on 5 additional uninjured rats treated with 0.3 mg/kg MK-801. Behavioral assessments were made for 5 days after injury. Treatment with 0.3 mg/kg MK-801 resulted in significantly less weight loss and significantly reduced beam-balance and beam-walking deficits. MK-801 did not significantly alter rectal or brain temperature. These data suggest that NMDA receptor interactions contribute to the pathophysiology of brain injury. Supported by NS 21458, NS 12587 and Merck Sharp & Dohme.

444.5 MK801 NEUROPROTECTIVE EFFECTS IN A RODENT MODEL OF PEPTIDE INDUCED SPINAL CORD INJURY: A HISTOLOGICAL STUDY. D.D. Rigamonti, A. Martinez-Arizala, R.F. Genovese, J.W. Holaday, and J.B. Long, Dept. Med Neurosci, Walter Reed Army Inst Res, Wash, DC 20303. DYN, a potent NMDA receptor antagonist, prevents the NMDA neurotoxicity associated with hindlimb paralysis, nociceptive loss and spinal cord ischemia when injected into the lumbar cistern. Since NMDA receptors have been implicated in the pathophysiology of ischemic CNS injury, we examined the effects of MK801, a non-competitive NMDA receptor antagonist, on DYN-induced histopathological changes. Adult S-D rats were divided and injected intrathecally as: Group I-saline, Group II-DYN, and Group III-DYN+MK801. After neurological evaluation for 72 hours, the rats were perfused, the lumbosacral cord was removed, and serial cross-sections were analyzed using H & E Nissl, Kliver-Barrera and Bielschowsky techniques. Samples were evaluated by three blinded observers. Interrater agreement and differences between groups were statistically analyzed. Spinal tissue from I appeared normal while tissue from II exhibited lumbosacral cord necrosis. The injury was characterized by widespread ischemic cell changes, neuronal cell loss and axonal degeneration in the gray and adjacent white matter, with diffuse gliosis and cellular infiltration. Tissue from III exhibited small islands of necrotic tissue or slight ischemic changes, restricted to the gray matter. In addition, there was mild gliosis and edema. Thus, MK801 improved neurological and neuropathological outcome in DYN-treated animals.

444.6 MUSCARINIC AND NMDA RECEPTOR BLOCKADE REDUCES POST-ISCHEMIC EGG SPIKE FREQUENCY FOLLOWING TBI AND ACUTE SECONDARY ISCHEMIA. L. Jenkins, B. Lyeth, D. DeWitt, R. Hamm, L. Phillips, H. Young, G. Clifton*, and R. Haves. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298. Following mild traumatic injury (TBI) subsequent brain vulnerability to cerebral ischemia is enhanced (Brain Research 477:311,1989) and receptor related since combined muscarinic and NMDA receptor blockade can significantly reduce enhanced delayed neuronal death after TBI followed by acute or delayed cerebral ischemia (J Neurotrauma 5:303,1988). We examined the effect of muscarinics and NMDA receptor blockade on the frequency of post-ischemic EEG spike activity as one possible mechanism for neuroprotection. Rats (n=250-300g) were subjected to mild TBI followed 1 hr later by 6 min of forebrain ischemia. Two groups (N=4/group) were examined: one without drug treatment and another group with simultaneous i.p. 1mg/kg scopolamine and 4mg/kg phenytoin (PCP) 15 minutes prior to TBI. Drug treated rats (21 ± 0.8 spikes/min) demonstrated a 276% reduction in post-ischemic spike frequency compared to the untreated group (81 ± 0.9 spikes/min). Regenerative neuronal spike activity and post-ischemic calcium entry (J Cereb Blood Flow & Metabol 8:799,1988) are known to be influenced by both NMDA receptor linked inward calcium currents and muscarinic receptor linked outward potassium currents (CNS 10:530,1987). Thus, neuronal protection by combined scopolamine and PCP in mild TBI followed by acute secondary ischemia may be related to receptor modulation of these Ca²⁺ and K⁺ channel protein conductance states. Supported by NS19950 and NS12587.

444.7 EFFECT OF PHENCYCLidine (PCP) ON CENTRAL CHolinergic ACTIVITY FOLLOWING TRAUMATIC BRAIN INJURY (TBI). S.P. Robinson, E.K. Enter*, M.G. Posner*, S.D. Fox*, R.M. Martin*, C.A. Gveyen* and R.R. Davis*, Dept. of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298-0613. TBI has been found to produce changes in acetylcholine (ACh) turnover at early times following injury (Baigia et al, Brain Res. 452: 303, 1988). Because PCP, a noncompetitive NMDA antagonist, reduces some of the behavioral deficits following TBI (Hays, R.L et al. Soc. Neurosci. Abst. 17:154, 1991), we examined the effect of PCP on TBI prostituted on ACh turnover in the dorsal hippocampus, frontal cortex, and caudate nucleus, areas containing cholinergic and excitatory amino acid innervation. TBI produced a significant increase in turnover rate (TRACCh) compared to sham-injured rats (2.1 ± 0.7%/min) in all areas (P<0.05). We found that activation of NMDA receptors may lead to changes in cholinergic activity following TBI. These changes may contribute to long-term behavioral changes that occur after TBI. (Supported by NS 24415 and NS 07288).

444.8 TRAUMATICALLY INDUCED BLOOD-BRAIN BARRIER DISRUPTION: A CONDUIT FOR THE PASSAGE OF CIRCULATING EXCITATORY NEUROTRANSMITTERS. J.T. Povlishock and B.G. Lyeth Depts. of Anatomy and Surgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298. In rodent, traumatic brain injury (TBI) results in the release of excitatory neurotransmitters that participate in pathological agonist-receptor interactions (Brain Res. 542:299,1991). It has been assumed that all excitatory neurotransmitters are derived from synaptic terminals, however, it is possible that some reach the brain via a compromised-blood-brain barrier (BBB). To test this, BBB status was assessed in brain regions previously linked with functional endothelial change. Rats were subjected to mild TBI and were processed for the immuno cytochemical detection of various brain neurotransmitter immunoglobulins, and neurotransmitters normally excluded by the BBB. TBI resulted in the resultation of immunoglobulins and excitatory amino acids such as glutamate into the interstitiates of all the cerebral cortices and dorsal hippocampi. These substances crossed the BBB without any overt endothelial change. Their passage was transient and interestingly was blunted by the administration of superoxide dismutase. Supported by NS-20193.
RAPID GENOMIC RESPONSE TO LOCAL INJURY IN HIPPOCAMPAL MORPHOLOGY ARE DISTINCTLY DIFFERENT FROM OLIVOCOCHLEAR DENTATE GRANULE CELLS. A.J. Cole, P.F. Worley, and J.M. Hamilton syringe injection frequently elicited an p<0.002); reticular formation (46.8 ± 21.3 vs 6.1 ± 10.0; p>0.05). However, counts of HRP-labeled cells were counted in the brainstem and motor cortex, regardless of the site of injury. These findings indicate that direct intracerebral injections may be insufficient to activate a neuronal genomic response. Moreover, this type of simple penetrating head injury offers a model system to elucidate the mechanisms of both local and hemispheric genomic responses.

THE EFFECT OF A DIRECT CURRENT FIELD ON CHRONICALLY INJURED MAMMALIAN SPINAL CORD AXONS. M.G. Pfeiffer, E.H. Totor (Spon: E. Theriault), Playfair Neurosci. Unit, Univ. Toronto M5T 2S8 Recent studies indicate that direct current (DC) fields promote the recovery of acute injured axons; however, the effect of DC fields on chronically injured spinal axons is unknown. In this study, 14 S/C rats (C57Bl/6J) with lesions produced by 28-gauge stimulating electrodes were divided into three groups: 12 weeks after injury, motoneurons and somatosensory evoked potentials were recorded and HRP was introduced into the T6 cord. Labeled cells were counted in the brainstem and motor cortex and axons were counted at the injury site. The inclined plane scores, evoked potentials, and axon counts of treated and control rats were similar (p>0.05). However, counts of HRP-labeled neurons were significantly higher in treated rats in the red nucleus (96.8 ± 29.5 vs 24.6 ± 7.5; p<0.002); reticular formation (46.8 ± 21.3 vs 6.1 ± 2.0; p<0.001) and raphe nuclei (83.8 ± 22.1 vs 15.8 ± 4.8; p<0.001). Thus, although ineffective in promoting recovery in chronic injuries, DC stimulation does upregulate retrograde axonal transport, as evidenced by increased labeling of neurons by HRP. This may be a mechanism by which DC fields promote recovery after acute injuries.

444.11 NON-OLIVOCOCHLEAR CHOLINERGIC PERIOLIVARY CELLS. J.C. Adams, Dept. of Otolaryngology and Comm. Sci., Med. Univ. of S.C. Charleston, S.C. 29425. A modified Koelle method was used to histologically localize esterase and in the same brainstem sections of cat, choline acetyltransferase was visualized using immunocytochemistry. This procedure presented a cochlear nucleus, and its projections. The present experiments also show that the esterases are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either.

444.12 HEAD INJURY AND BRAIN ENERGY CONTENT: AN IN VIVO ASSESSMENT BY INTEGRATED MRI/3-P MAGNETIC RESONANCE SPECTROSCOPY. M. Range**, B.S. Jenkins**, T.G. Donnelly**, J.A. Allen**, M. C. C. Holzen**, R.L. Grossman**(Spon: L. B. Saunders). Dept. of Neurology, Radiology, and Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104. We have studied the content of brain phosphorus compounds in humans after acute head injury. 13 patients were evaluated at a mean interval of 14 days after trauma and follow-up examinations (more than 30 days after injury) were carried out in eight of these cases. Most of the patients were on assisted ventilation. All MRI/3-P studies were performed on a 1.5 T MR imaging system with the standard spectroscopic research accessory. Dose localization was employed in eight cases and a 3D-CI sequence which yielded spectra from both voxels in the others. Spectra were processed off-line using a computer program written specifically for the analysis of in vivo spectra. In large focal insults the PCr/PI ratio was reduced in the affected side of the brain. Moreover, in some of these cases little or no observable ATP resonances were detectable. Abnormal spectra in hyperventilated patients exhibited a more normal appearance when the patients were returned to unassisted ventilation. It is not clear at this point, whether these changes are a reflection of a recovering brain function or are a direct result of the ventilation state of the patient. We are currently extending our studies into the first 48 hours.

AUDITORY SYSTEM

445.1 NON-OLIVOCOCHLEAR CHOLINERGIC PERIOLIVARY CELLS. J.C. Adams, Dept. of Otolaryngology and Comm. Sci., Med. Univ. of S.C. Charleston, S.C. 29425. A modified Koelle method was used to histologically localize esterase and in the same brainstem sections of cat, choline acetyltransferase was visualized using immunocytochemistry. This procedure presented a cochlear nucleus, and its projections, as well as the two enzymes are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either.

445.2 ELECTRICAL STIMULATION OF THE INFERIOR COLICULUS AT LOW RATES PROTECTS THE COCHLEA FROM AUDITORY DESENSITIZATION. R. Jakoby*, S.P. Smith*, B.F. Irvine. Department of Physiology, University of Western Australia, WA 6009, Australia. Electrical stimulation of the inferior colliculus (IC) contralateral to a cochlea presented a cochlear nucleus, and its projections, as well as the two enzymes are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining compared with cranial motor nuclei. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show that the esterases are present in all cells that show the presence of either.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
445.3 SHAPE, SIZE, AND ORIENTATION OF THE DENDRITES OF THE COCHLEAR NUCLEUS NEURONS IN GUINEA PIG. L. L. Terr, W. Pimpinellaion,* and J. K. Moore. Lab. of Neuroanatomy, House Ehr Institute, Los Angeles, CA, and Dept. of Anatomical Sciences, SUNY at Stony Brook, NY.

This report provides quantitative and qualitative data on three-dimensional projections of the dendrites of cochlear nucleus neurons and also qualitative 3-D study of them in three guinea pigs. The sections were from tissue blocks stained by the Golgi-Hortega technique and embedded in celloidin. A neuron tracing computer system to reconstruct the shape of the neurons as revealed by metallic impregnation was used. Dendrites and soma contours were traced and entered into a computer with a vector display processor. To characterize the reconstructed neurons as bushy, stellate, giant, granular, or octopus, we evaluated the following: total length of the dendrites; volume of the neurons, including the distal part of the dendrites; cross-sectional area of the soma; number of main dendrite trees originating from the soma; number of the dendritic appendages; and number of branch points. The performed statistical analysis to determine the means and standard deviations of these parameters. We also studied the orientation of the dendrites in relation to the surface of the cochlear nucleus and the root of the cochlear nerve. Findings could be applied to anatomical studies of the pathological changes of dendritic networks in deafness or to physiological studies of the cochlear nucleus. A mathematical neuron model could be made to explore such problems as the relation between the time course and the soma–dendritic location of synaptic input.


We conducted population studies of neurons of the posteroventral and dorsal cochlear nucleus (PCVN & DCN) and the auditory nerve fibers in decerebrate cats in investigating responses to vowels [a] and [e], and pure tones. The goal is to elucidate how these signals are encoded in the auditory nerve fibers and how they are further processed in the PCVN and DCN. Several types of response profiles are computed: (1) driven discharge rate vs individual neuron’s characteristic frequency (CF); (2) d’ vs neuron’s CF, where d’ is a measure of detectability of rate increase; (3) a vector signal detection theory (3) spatially-weighted Fourier components of temporal discharge patterns vs neuron’s CF a ‘la Young & Sachs’ (1979) “ASLR.” We find that the response profiles derived from the mean driven rate and d’ are generally similar. Our data indicate that the PCVN and DCN neurons’ response profiles are different from those of the nerve fibers as follows. The response profiles derived from the driven rate of the primary nerve fibers with high spontaneous rates are severely degraded at 70 dB SPL by saturation of discharge rate limited to about 200 spikes/sec. In contrast, the PCVN and DCN neurons’ response profiles are little affected by rate saturation. Some of the PCVN and DCN neurons’ discharge rates reached 400-500 spikes/sec at 70 dB SPL. Our observations support the hypothesis that the PCVN and DCN neuronal mechanisms transform the primary nerve fiber representations of the vocal spectrum into enhanced representations. We postulate that a possible such mechanism is lateral inhibition in the cochlear nucleus. (Supported by a grant NS 52363 from NIH and grants from Univ. Conn. Health Center)


One way in which animals localize sound in the azimuth is the intensity differences at the two ears. The neurons in the lateral superior olive (LSO) encode this cue by integrating the synaptic drive from ipsilateral excitatory and contralateral inhibitory connections. This synaptic integration has been analyzed in 500 urn neurons in the LSO and the dorsal nucleus of the trapezoid body (MNTB) by evoked EPSPs and IPSPs to encode interaural intensity. The results confirm that the LSO integrates evoked EPSPs and IPSPs to encode interaural intensity.

445.6 SELECTIVE LOSS OF GABA NEUROTRANSMISSION IN THE INFERIOR COLLICULUS OF AGED FICHER-344 RATS. A. Raza* and S.P. Arneric. (SPON: W.H. Cline), Department of Pharmacology, Southern IL. University School of Medicine, Springfileld, IL 62702.

Immunocytochemical studies have suggested an age-related loss of GABA-containing cells in the central nucleus of the inferior colliculus (ICC), a critical auditory nucleus (Caspari & Lawhorn, Neurosci. Abstr.13:150, 88). This study was designed to determine: 1) the density of GABAergic sources of ICC; 2) if so, is the ICC GABA impairment regionally specific? Amino acid concentrations were determined by HPLC. Release of endogenous GABA, glutamate (Glu), aspartate (Asp) and TH-aminohexanoyl (TH-Ahx) was measured from micropatches (1 mm x 0.5 mm thickness) of the ICC and compared with rostral ventrolateral medulla (RVM) and somatosensory cortex (SSC) in young (7-17 months) and aged (24-26 month) Fischer-344 rats. GABA neurotransmission is involved in processing sensory information in these regions. Depolarization with 35 mM K+ evoked release of GABA (54%), Glu (12%), Asp (23%), and TH-Ahx (90%) which was substantially Ca2+-dependent (n = 12, p < 0.05). Basal and K+-evoked release of GABA in the ICC of aged rats was reduced to 32-43% of young, (n = 8, p < 0.05), while other transmitters were unaffected. In addition, there was a corresponding % reduction in tissue levels of GABA (p < 0.05) while other amino acids were unaffected. This age-related GABA deficit was not observed in RVM or SSC. CONCLUSIONS: 1) These data support the idea that GABA, Glu, Asp and ACB serve a neurotransmitter role in the ICC; 2) These results support and extend the finding of Caspari & Lawhorn (1987) that GABA neurotransmission is selectively impaired in ICC of aged Fischer-344 rats; 3) Loss of inhibitory neurotransmission in ICC may be one of the neurochemical correlates to the deficits in auditory perception associated with presbycusis. (Supported by the Central Research Committee, Southern IL. Univ. School of Medicine and NSA N01-AU-21040)


Previous experiments using retrograde degenoration and anterograde transport of tritiated amino acids method have shown that a very neurologically primitive mammal, Mammal domesticus, less than 1% of the cells in the MG project to the IC while 7.4% project to the dorsal nucleus of the inferior colliculus (DNC). Conventional receptor binding techniques show that the density of GABA receptors in MG parallels this caudal-rostral difference in subcortical projections while nACh and glycine receptors do not. However, anti-GABA immunocytochemistry reveals very few (<1%) GABAergic neurons in MG and these are scattered throughout its rostral-caudal extent. This mismatch of intrinsic GABAergic neurons and GABA-A receptors suggests that most of the GABA-A receptors in caudal MG, those modulating the subcortical projections, may be accommodating extrinsic sources of GABAergic input. Supported by NIH-NINDB # NS17726.

445.8 GLYCINERGIC AND GABAERGIC AUDITORY BRAIN STEM NEURONS AND AXONS IN THE MUSTACHE BAT. G.D. Pollak and J.A. Whiten. Dept. of Zoology, University of Texas, Austin, TX 78712, and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Neurochemically distinct pathways were studied in the bat, Pteronotus parnellii, with antiseria directed against glycine conjugated to glutaraldehyde or glutathione and guanidinoacetic acid (GABA) to identify neurons and axon terminals (puncta). There are four main findings. The first is that the medial nucleus of the trapezoid body (MNTB) has predominant glycine-containing neurons. The second finding is that there is a pronounced gradient of increasing immunoreactivity along the rostral-caudal axis of MNTB, i.e., along an isofrequency contour. There is also a gradient of GABA axons and puncta along the rostro-caudal axis of the lateral superior olive (LSO). Since the LSO is a direct projection from the MNTB, it is possible that these gradients establish an orderly distribution of inhibitory thresholds of E-I neurons in LSO. The third finding is that the inhibitory network is characterized by the predominance of their immunoreactivity. The MNTB is predominantly Gly+, whereas the LSO and dorsal nucleus of the lateral lemniscus are predominantly GABA+, as are inferior colliculus cells. The fourth finding is that distinct Gly+ and GABA+ axonal fascicles can be traced from the trapezoid body into the 60 kHz contour of the mustache bat’s inferior colliculus. The third finding is that several of the descending inhibitory neurons of the MNTB are characterized by the predominance of their immunoreactivity. Supported by ROI NS 22766-05 (GDP) and NS 22766-09 (J.A.W). We thank R. Wenthold for antiserum to glycine and GABA.
INHIBITION SHAPES RESPONSES TO INTERAURAL LEVEL DIFFERENCES IN THE INFERIOR COLLICULUS OF THE BARN OWL.

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Terry T. Takahashi, Institute of Neuroscience, University of Oregon, OR 97403.

Barn owls use interaural differences in sound pressure level (ILD) to compare location of the source in elevation. The external nucleus of the inferior colliculus (ICx) maps auditory space. Elevationally restricted auditory receptive fields are coded for by non-monoaminergic neurones in ICx. ICx polysynaptic input from lower brainstem. Information about ILD is carried in an anatomically and functionally distinct pathway into ICx. Important lower brainstem nuclei include nucleosi angularis and nucleus ventralis lateralis, pars posterior (VLv).

We injected the local anesthetic lidocaine hydrochloride focally into VLv while recording neuronal responses to varying ILD in the contralateral ICx. VLv was found to contribute functional inhibition at ILD's favoring the ear ipsilateral to the side on which responses were recorded in ICx (shown schematically in the figure). This result is consistent with the known hodology and with the functional response properties of VLv. Preliminary results support the hypothesis that this inhibition is established directly by VLv in the lateral shell of the central nucleus of the inferior colliculus.

R.A. is a Howard Hughes Medical Institute Fellow.

INHIBITION OF IDENTIFIED LOSTER SEROTONERGIC NEURONS.


Inhibition plays an important role in the control of behavior. Results in several systems suggest that inhibitory neurones suppress certain behaviors, and a decrease in activity of such neurones may release previously inhibited behaviors. Our recent efforts have focused on the role of the inhibitory system in the song system of the male zebra finch. In this system, the motor output is mediated by GABAergic neurons that express a high level of the GABA transporter, GAT-1.

We have recorded from single GABAergic neurones in the song system of the male zebra finch and found that the inhibitory system is composed of two distinct classes of neurones: (1) those that are excited by GABAergic input and (2) those that are inhibited by GABAergic input. The latter class of neurones is composed of two distinct subpopulations. The first subpopulation is composed of neurones that are excited by GABAergic input and are involved in the production of the song. The second subpopulation is composed of neurones that are inhibited by GABAergic input and are involved in the production of the song.

We have also recorded from single GABAergic neurones in the song system of the male zebra finch and found that the inhibitory system is composed of two distinct classes of neurones: (1) those that are excited by GABAergic input and (2) those that are inhibited by GABAergic input. The latter class of neurones is composed of two distinct subpopulations. The first subpopulation is composed of neurones that are excited by GABAergic input and are involved in the production of the song. The second subpopulation is composed of neurones that are inhibited by GABAergic input and are involved in the production of the song.
CHARACTERIZATION OF SPECIFIC EARLY PROTEINS INDUCED BY 5-HT AND CAMP DURING ACQUISITION PHASE OF LONG-TERM FACILITATION IN APLYSIA SENSORY NEURONS. A. Barzilai, T.E. Kennedy, J.D. Sweet and E.R. Kandel. HHMI. Columbia, NY, NY 10032.

Induction of long-term memory for sensitization and long-term presynaptic facilitation by 5-HT and CAMP in sensory neurons is dependent on active transcription and translation during the initial sensitization phase, the period during which 5-HT is present. To gain insight into these long-term events, we have focused initially on the earliest detected transcriptional changes induced by 5-HT in Aplysia neurons, which occur within 1-3 hours. These changes are transient, and subside within 1 to 3 hours. The same proteins were also induced by CAMP, suggesting that the induced genes might bear a cAMP recognition element. In these several features - rapid and transient induction, transcriptional dependence, and requirement for a second messenger - these proteins resemble the immediate early gene products induced in vertebrate cells by growth factors. To test this idea and to explore the regulatory and effector roles of these proteins in the induction of long-term sensitization, we have obtained partial amino acid sequence of three Aplysia early proteins using the method of Kennedy et al. (Science 269, 1995) in an attempt to obtain cDNA clones corresponding to the proteins.

REFERENCES

1. Barzilai, A., Barzilai, J.E., D. Sweet and E.R. Kandel. HHMI, Columbia, NY, NY, 10032. Maintenance of long-term sensitization in Aplysia is associated with a sustained increase in the incorporation of [35S]-methionine into proteins. Two of these proteins, #407 and #1403, show a similar change of expression in isolated clusters of sensory neurons where 5-HT, rather than tail shock, is used to produce long-term facilitation. The expression of these proteins is blocked by inhibitors of macromolecular synthesis present during the period of 5-HT application. We have cloned the cDNA corresponding to protein #407 using partial amino acid sequence, and found no significant homologies in sequence data bases. By contrast, partial amino acid sequence, pl and M.W., indicate that protein #1403 is the Aplysia homologue of GRP77/BiP. Aplysia BiP also changes its expression in pleural sensory neuron clusters following application of CAMP or heat shock to 37°C. BiP is thought to function as a chaperon of newly synthesized proteins in the lumen of the ER. This is consistent with the finding that Aplysia BiP first increases in expression at 3 hours following 5-HT application when overall protein synthesis is maximal (Barzilai et al. 1989). Using PCR, we have obtained a partial cDNA clone of Aplysia BiP and are examining the distribution and expression of both of the late protein following long-term training.

446.5


Maintenance of long-term sensitization in Aplysia is associated with a sustained increase in the incorporation of [35S]-methionine into four proteins. Two of these proteins, #407 and #1403, show a similar change of expression in isolated clusters of sensory neurons where 5-HT, rather than tail shock, is used to produce long-term facilitation. The expression of these proteins is blocked by inhibitors of macromolecular synthesis present during the period of 5-HT application. We have cloned the cDNA corresponding to protein #407 using partial amino acid sequence, and found no significant homologies in sequence data bases. By contrast, partial amino acid sequence, pl and M.W., indicate that protein #1403 is the Aplysia homologue of GRP77/BiP. Aplysia BiP also changes its expression in pleural sensory neuron clusters following application of CAMP or heat shock to 37°C. BiP is thought to function as a chaperon of newly synthesized proteins in the lumen of the ER. This is consistent with the finding that Aplysia BiP first increases in expression at 3 hours following 5-HT application when overall protein synthesis is maximal (Barzilai et al. 1989). Using PCR, we have obtained a partial cDNA clone of Aplysia BiP and are examining the distribution and expression of both of the late protein following long-term training.

446.6


Short-term facilitation (STF) of Aplysia sensory-motor synapses (as induced by a 2 minute application of 5-HT) differs from long-term (24 hour) facilitation (LTF) (as induced by a two hour application of 5-HT). In both cases, protein synthesis increases in both levels of phosphorylation and rate of synthesis. To study the basis of this macromolecular synthesis dependence we are characterizing proteins that have been shown by Barzilai et al. (Neuron, 1989) to change in their rate of synthesis during the acquisition phase of LTF. One of the first proteins to go up in synthesis (or a protein that migrates to the same spot on SDS-PAGE) also increases in its level of phosphorylation after two hours of 5-HT treatment. This delayed phosphorylation increase is distinct from the previously described, more rapid phosphorylation changes that occur in 17 other proteins (Sweet and Kandel, Nature, 1989). Preliminary subcellular fractionation studies suggest that this protein is present in the nucleus. Perhaps this protein contributes to the switch from STF to LTF by detecting the 5-HT-stimulated kinase activity at a critical period after the protein’s synthesis. Alternatively, the increase in phosphorylation might simply reflect the increase in the protein’s mass. To further characterize this protein, we have obtained partial amino acid sequence and are now attempting to clone the corresponding cDNA.

446.7


Aplysia neurons have two forms of PKC that can be distinguished by their response to arachidonic acid (AA). One activated by high concentrations of AA (similar to vertebrate β) and the other at low concentrations (analogous to γ). In Aplysia neurons, a phorbol ester (5-HT or phorbol ester) activates PKC, translocating the kinase from cytosol to membranes. The membrane-associated PKC, a 29,000 protein (Calignano, Pomelli, Wallner & Schwartz, Neurosci. Abstr. 1988, 14:111), when phosphorylated by PKC through either phorbol ester or 5-HT, activates PLα2 to produce AA. AA continues to stimulate PKC after the translocation second-messenger signal for the initial translocation is dissipated, thereby maintaining the kinase in an active form to enhance synaptic strength persistently: the cycle is thus completed and continued. This mechanism for persistence is relevant for models of learning and memory where PKC plays an important role: LTP in hippocampus, conditioning of the rabbit eye blink response, and sensitization of defensive reflexes in Aplysia.

446.8

MEMORY MUTATIONS AFFECT AN ELEMENTARY EXPERIENCE-DEPENDENT MODIFICATION OF RESPONSE IN AN IDENTIFIED SENSORY NEURON OF DROSOPHILA. G. Carls*, and Y. Duda. Dept. of Neurobiology, Weizmann Institute, Rehovot 76100, Israel.

Stimulation of the thoracic bristles of Drosophila elicits a cleaning reflex. The habituation of this reflex is defective in two memory mutants: rut and cs. Rut lacks the Ca2+ and cAMP activated adenylate cyclase, and cs, which has a reduced cAMP phosphodiesterase (PDE) activity. We have now investigated the physiology of a sensory neuron that subserves this behavior. Extracellular recordings were performed from truncated bristles. The recording electrode was also used for mechanical stimulation. We have studied two plastic processes in the sensory neuron: the decrement of response to a sustained stimulus (adaptation), and the decrement of response to repetitive stimuli (sensory fatigue). The mutations rut and cs result in increased sensory fatigue. rut, cs, and mutants lacking both rut and cs, show an increased sensory fatigue. rut, mcw sensory neurons fatigued slower and dsc neurons fatigued faster than normal. The opposite effect of the rut and dsc mutations on sensory fatigue suggests that cAMP is cAMP dependent. To test this hypothesis we investigated the effect on sensory fatigue of systemic injection of drugs which affect the cAMP cascade. PDE inhibitors caused rapid increases in wild-type (CS) neurons, mimicking the effect of dsc. In contrast, rut neurons were not affected by PDE inhibitors. The adenylyl cyclase activator forskolin decreased sensory response in naive CS and rut neurons. The protein kinase inhibitor H7 reduced the effect of repetitive stimulation in CS and dsc neurons. Our results indicate: a. the CS cascade is involved in sensory fatigue; b. memory mutations affect an elementary form of neuronal plasticity.

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MULTISECOND AFTERDISCHARGE OF SPINAL CORD INTERNEURONS EVOKED BY CUTANEOUS STIMULATION IN THE ROSTRAL SCRATCH RECEPTIVE FIELD OF THE TURTLE.

Scott N. Currie and Paul S.C. Stein. Dept of Biology, Washington University, St. Louis, MO 63130.

Stimulation of cutaneous afferents of the turtle increases the excitability of the spinal sensory neurons for several seconds following stimulus-offset (J. Neurophysiol. 60: 2122-2137, 1988), to which we have recorded from single cutaneous afferents and sensory interneurons in order to examine the neuronal mechanisms for multisecound increases in spinal excitability. A single segment of the midbody spinal cord was isolated in situ by transsecting the cord at the segment's anterior and posterior borders. The isolated segment was left attached to its peripheral nerve that innervates part of the rostral scratch receptive field. A micro suction electrode was used to record from descending axons in the white matter. We identified a subset of sensory interneurons that exhibit a multisecound afterdischarge for as long as 10-30 seconds following mechanical or electrical stimulation to the rostral scratch receptive field. These cells displayed long-latency depolarization in response to single electrical pulses delivered to the shell at multisecound intervals. Cutaneous interneurons with long latencies innervated the central nervous system for multisecound excitability changes in the spinal network for scratch reflex. Supported by NIH Grants NS07057 to S.N.C. and NS15049 to P.S.C.S.

A PERIPHERAL FEEDBACK LOOP MEDIATED BY NEUROMODULATORY SEROTONERGIC AFFERENTS COUPLES TWO CPGS IN A PHASE-INDEPENDENT FASHION. P.S. Katz and R.M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In the foregut of the crab, Cancer borealis, serotoninergic mechanosensory afferents, called GPR cells, controls pyloric cycles. The GPR cell innervates the pyloric network in a manner that is mimicked by serotonin application. The effects of GPR stimulation on these cells are mediated by discrete fast synaptic potentials and are therefore transient. In the crustacean pyloric system, GPR stimulation does not alter the pyloric network. Therefore, the effects of serotonin on GPR stimulation may be mediated by a peripheral feedback loop. These observations and previous results support the hypothesis that serotoninergic feedback is involved in the pyloric network. Supported by NIH NS17323 and Hatch Act Grant 19140.

Spiking cells of the central pattern generator (CPG) for the pyloric motor pattern in the stomatogastric ganglion of the spiny lobster *Panulirus interruptus* use graded synaptic transmission (electrical coupling and chemical inhibition) to coordinate their rhythmic activity. We examined the temperature dependence of graded synaptic transmission between identified pyloric CPG cells in STGs isolated from animals maintained at 15°C. Synaptic input-output curves for graded transmission were gathered in 10°C by measuring the postsynaptic peak polarizations resulting from 1 sec presynaptic polarizations of varying sign and amplitude. As the temperature was lowered from 23 to 10°C, graded chemical synaptic potentials showed reductions in peak amplitudes of approximately 55% to 100% between different cell pairs. These reductions in graded chemical synaptic strength were not accounted for by general membrane resistance decreases; they were accompanied by increased antidromic action potential size in both presynaptic and postsynaptic cells, increased presynaptic soma input resistance, and increased electrical coupling between some presynaptic cells. These reductions occur within natural temperature ranges for the spiny lobster (12-21°C), suggesting that the balance of synaptic interactions producing this motor rhythm may be a dynamic function of environmental temperature. Supported by NRSAS 157859 (BRJ) and NIH grant #5157323 (RMW-H).

447.5


Preliminary work (Hooper and Weiss, Soc. Neurosci. Abst., 1988) has shown that crab dialation, a known salination signal, excites certain baccal ganglion neurons. We therefore chroniclly implanted extracellular microelectrodes on various baccal neurones to monitor baccal ganglion activity during feeding and salination. These recordal suggest that the baccal ganglion can produce an unusual rhythmic pattern that has a characteristic and generally external behavioral correlate. The pattern consists of organized bouts of spiking activity lasting 1.0 to 2 min that repeat in a manner with other baccal patterns from known baccal ganglion cell types (blinking, swallowing, rejection). In these patterns, the spiking activity in these patterns lasts only 5 to 10 sec. These data suggest that the baccal ganglion may give rise to several patterns that are characterized by a repeated occurrence of a brief spiking phase, each of which may be a function of environmental temperature. Supported by NIH grant NS07859 (BRJ) and NIH grant #5157323 (RMW-H).

447.6

AN IDENTIFIED INTERNEURON TERMINATES PATTERED MOTOR ACTIVITY IN THE BACCAL GANGLION OF HELISOMA, A.D. Murphy, and F. Mehdidi-Hamadani*, Dep't of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60607.

Patterned motor activity (PMA) underlying feeding in the snail, *Helisoma*, must be regulated by descending inhibition from the "brain" (circumoesophageal ganglion's ring). The baccal ganglia (BG) contain most of the interneurons comprising the central pattern generator that mediates feeding. The BG are connected to the brain via the cerebral-buccal commissures (CBC). Severe lesions of the CBCs or applying a procaine block to the CBCs in a semi-intact animal typically activates rhythmic "feeding" movements. To localize putative inhibitory interneurons in the brain, we used retrograde staining of cut axons in the CBC using Lucifer Yellow. This revealed a neuron with a soma in the pleural ganglion and an axon that traverses the pleuro-oesophageal connective and the CBC. Subsequent in vivo recordings confirmed that this neuron, PLBII (pleuro-buccal inhibitory neuron 1), is a potent inhibitor of PMA in the BG. A train of action potentials in PLBII terminates PMA even in the presence of 10-6 M serotonin. Serotonin is an excitatory neuromodulator which induces vigorous PMA at that concentration. Intracellular injection of PLBII with Lucifer Yellow CH revealed a neuritic arbor in the pleural ganglion and axon branches arborizing in the ipsilateral parietal ganglion, the ipsilateral pedal ganglion, and the ipsilateral cerebral ganglion. The main axon traverses the ipsilateral CBC and innervates both bucral ganglia, crossing the buccal commissure. (Supported by NIH Grant 1 ROI NS26443-01).

447.7

CONNECTIONS OF IDENTIFIED INTERNEURONS IN THE LEECH ARISE IN NEURAL NETWORKS TRAINED BY BACK-PROPAGATION, S.R. Lockery, G. Wittenberg, W.B. Kristan Jr., and T.J. Segundo, U.C.S.D. and Salk Institute, La Jolla, CA 92037.

Interneurons in model networks generated by the back-propagation algorithm were compared to identified interneurons in the local reflex circuitry of the leech. Touching dorsal, ventral, or lateral skin causes a localized withdrawal from the stimulus (local bending). Four mechanoreceptors (P cells) with dorsal or ventral receptive fields provide the models connect to eight types of dorsal and ventral motor neurons via interneurons, of which nine types have been identified. Strengths of P cell to interneuron connections were measured as a function of peak synaptic potential to the segmental cell impulse train. Output connection strengths for two interneurons were measured as the amplitude of synaptic potentials in the motor neurons following P cell depolarization of the interneurons. We trained a model network of four sensory, eight motor, and nine pairs of interneurons using back-propagation to reproduce the amplitudes of synaptic potentials produced by the three types of local bending. The network included known lateral connections among motor neurons, but no constraints were placed on the pattern of interneuronal inputs and outputs. However, following training the model interneurons, like the real ones, received inputs from dorsal and ventral sensory cell types, and connected to all eight motor neurons. Additional biological constraints, including recurrent connections and temporal response properties, can now be used to improve the predictions of the model. Supported by NIH research grant NS25916 and the Bank of America—Giannini Foundation.

447.8

INTERSEGMENTAL INTERNEURONS contribute to multiple behaviors in the leech, G. Wittenberg, S.R. Lockery, and W.B. Kristan Jr., Dep't of Biology, Univ. of California at San Diego, La Jolla, CA 92037.

Electrophysiological studies in the CNS of the leech, *Hirudo medicinalis*, were performed in order to define and understand the production of particular behaviors. When the leech receives a mechanical stimulus, a patterned motor activity (PMA) is produced in a series of motor patterns, three of which are local bending, swimming, and shortening. Local bending is a segmental withdrawal response produced by excitation of motor neurons (MNs) innervating longitudinal muscles and inhibition of MNs innervating muscles on the opposite side. Swimming involves the entire body and is produced by antipodal rhythmic excitation and inhibition of the same longitudinal MNs. Shortening is a graded response, involving a few to several segments anterior and posterior to the site of stimulation; shortening is produced by excitation of all longitudinal MNs in that region. MNs of one anterior segment contribute to shortening as an unfacilitated lesion to the interganglionic commissures cause only a partial decrement in the response of segments distal to the lesion. These MNs which mediate local bending and swimming behavior have been identified and characterized. The present study used intracellular recording and stimulation of these and other MNs in an isolated CNS to begin to study their responses and effects in shortening behavior.

Local bending MNs and swim MNs both contribute to shortening behavior. Cell 11f, which participates both in local bending and swimming, produces excitation of dorsal longitudinal MNs two segments posterior to the segment in which it is located, in addition to receiving inputs from both dorsal and ventral sensory cells. Cell 20f, a swim IN, has the same motor effect, but receives a high, variable sensory input. In addition, previously unidentified MNs participate in shortening. One of those causes excitation of dorsal MNs, but inhibition of ventral MNs, two ganglia anterior to the segment in which it receives a dorsal sensory input. It appears likely that swimming, in addition to being produced by a single IN dedicated to the task, but by a network of MNs, some of which do not have input-output functions similar to the overall behavior. Supported by NIH research grant MH43396 and PHS training grant GM07198.
CHEMICAL DEAFFERENTATION OF INSECTS BY THE ALPHA ADRENERGIC ANTAGONIST PHENTOLAMINE.


A common approach for elucidating the function of sensory feedback in the organization of the motor activity is to remove afferent input and analyze the subsequent changes in the motor pattern. Usually deafferentation is done by transection of afferent fibers. This method is often technically complicated and usually cannot be done while maintaining records from single units. Alternatively, the drugs to induce the activity of sensory organs. We found that the α-adrenergic antagonist Phentolamine inactivates sensory organs in insects. When injected into the flight behavior of the locust it can be demonstrated that Phentolamine treatment results in a motor pattern very similar to the deafferented motor pattern as observed after mechanical deafferentation.

The inactivation of sensory organs is not due to an anesthetic action of Phentolamine on the peripheral sensory nerves, since spikes are conducted if electrically evoked. Phentolamine used at concentrations just sufficient to inactivate sensory organs does not abolish afferent activity and muscle activation and has very little effect on the CNS. However, high concentrations of Phentolamine blocked nerve conduction. The inactivation of sensory organs without affecting the CNS was specific for α-adrenergic antagonists, such as Phentolamine and Tolasoline α1-adrenergic antagonists (e.g. Yohimbine), β-adrenergic antagonists (e.g. Propranolol), 5-HT antagonists (e.g. Verapamil) or local anesthetics (e.g. Lidocaine) could also inactivate sensory organs, but only at concentrations which also had a strong central effect.

(Supported by the MRC of Canada).

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CELLULAR AND MOLECULAR STUDIES III

448.1

FREEZING LESIONS OF THE NEWBORN RAT BRAIN: A MODEL FOR CEREBROCORtical MICRODYSENSES.


Freezing lesions of the neocortex of newborn rats have been suggested as a model of cerebrocortical microdysgenesis, specifically microgyria and associated neurones. Freezing the cerebral cortex with freezing and infolding of the cortex to form a pseudo-sulcus, and accompanying disruption of cortical lamina, thereby replicating the results of Dvorak and colleagues. Longer exposure times and lower temperatures resulted in more severe lesions. In the depth of the pseudo-sulcus, four distinct layers were seen which could be the result of (1) laminar necrosis and associated neuronal migration to the underlying cerebral cortex, with thinning and infolding of the neocortex, and (2) disturbance of neuronal migration abnormalities and lead to significant focal cortical dysplasia.

These results suggest that early focal cortical injury may mimic neuronal migration abnormalities and lead to significant focal cortical dysplasia.

This work was supported, in part, by NIH Grant 20806, and by the Oron Dyslexia Society.

448.2


Freezing lesions to the left somatosensory neocortex of newborn rats were performed for periods ranging from 3-10 seconds and with temperatures ranging from -70°C to -110°C. At 60 days of age the animals underwent corpus callosumotomy. After one week, the animals were sacrificed, their brains removed, sectioned coronally, and processed for silver staining of degenerating axon terminals.

These lesions resulted in substantial focal neuronal loss and laminar dysplasia in the underlying cerebral cortex, and a pattern of transcallosal axonal terminals was disturbed in the surrounding regions. That is, although transcallosal axonal terminations are rare in somatosensory cortex, there was a pattern of dense terminal degeneration in portions of somatosensory cortex adjacent to the lesions produced by freezing. This is in direct contrast to a paucity of axonal terminal degeneration in the homologous area of the opposite hemisphere, and in analogous regions of control brains. This pattern of cortical axonal degeneration is different from that previously reported in an area of spontaneous cerebrocortical microdysgenesis in the rat and may reflect differences in the etiology (cause or timing) of the cortical disruptions.

We have demonstrated that freezing lesions of the neocortex performed in the newborn rat during the later stages of neuronal migration lead to an alteration in callosal connections in the surrounding regions.

This work was supported, in part, by NIH Grant 20806, and by the Oron Dyslexia Society.

448.3

COMPARATIVE NEUROGENESIS OF PEPTIDE CONTAINING CELLS IN NEOCORTEX OF RAT. M.E. Cavanagh* and J.G. Parnavelas*.

Brain Research Association*. Dep. of Anatomy, University College London, London WC1E 6BT, U.K.

Immunochemistry, to identify somatostatin (SRIF) Neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) containing cells, was combined with [3H]thymidine autoradiography (ARG) to determine the development of neurons. Pups were sacrificed at 4 weeks of age. PAP immunochemistry was performed on 10 μm sections cut at 0°C. Pups were injected with [3H]thymidine on days E14-E22. Pups were fixed from birth to 5 weeks of age. This work was supported, in part, by NIH Grant 20806, and by the Oron Dyslexia Society.

448.4


Organotypic slice cultures offer the possibility of studying the development of neurons in the absence of their specific extrinsic afferents. The combined Golgi/electron microscopy technique and glutaamin decarboxylase (GAD) immunocytochemistry were used to study the differentiation of pyramidal and GABAergic nonpyramidal neurons in slice cultures taken from 4-6 day old rats and mice.

Golgi-impregnated cultures revealed major cell layers of the hippocampus. Astersomatic contacts on gold-toned pyramidal cell dendrites and spines, normally occupied by extrinsic axon terminals, suggest synaptic contacts on GAD neurons. In fact, intrinsic mossy fibers and terminals of intrinsic GAD-positive neurons form synaptic contacts.

Our results demonstrate that hippocampal neurons develop their cell-specific characteristics, including characteristic synapses, in the absence of extrinsic afferents.

This study describes the sequence of interactions involved in developing vagal afferents and efferents and their neurochemically identified central targets in the brain stem in order to better define the role of structural and neurochemical abnormalities in the brain stems of SIDS victims in the pathogenesis of this disease. Neonatal Sprague Dawley rats from 0-14, 21 and 39 days old were used. Three types of experiments were performed on littersmates: 1) tracing vagal projections with antiserum directed against catecholamine synthesizing enzymes, SHT and selected peptides; 3) ultrastructural examination of vagal nuclei with and without labeling #1 and #2 above. 1) Thy and postganglionic nerve was injected with the help of chloral hydrate and horseradish peroxidase. 2) 10 neuronal profiles were perfused at each age for immunocytochemistry (Kalia et al., '85). In view of the small size of the brain stem, serial 50 um sections of each brain were taken in only one animal so that important rostral caudal levels were not missed. 3) Brain stems of littersmates perfused for electronmicroscopy were stained, stained, osmicated and embedded in plastic for ultrastructural examination. At 3 vagal projections showed the most proliferation but terminals and neurons in the medulla were only weakly immunoreactive. By day 10 the density of vagal projections was greatly reduced but still not as reduced as in the adult. Immunoreactive neurons and terminals showed a greater dispersion and lag behind developing vagal fibers during the period of early development. Supported by a grant from the SIDS Alliance.


Transgenic mice carrying multiple copies of the HOX-A-1 gene develop congenital megacolon and express elevated levels of Hox-A-1 mRNA in the gut. Megacolon also develops in mice because of decreased neural and glial precursors fail to colonize the terminal bowel. The terminal bowel of adult transgenic mice was found to be hypoganglionic and not aganglionic, like that of Drosophila. The ganglia and nerves in the hypoganglionic zone of the transgenic gut had the ultrastructure of extrinsic peripheral nerve, rather than that of the enteric nervous system (ENS). Scattered neuronal profiles in nerve bundles. In contrast to normal enteric ganglia, these neurons, as well as the nerves in which they were found had a giant collects containing enteronidium; supporting cells were surrounded by basal laminae, often myelinated axons, and thus are Schwann and not enteric glial cells. An abnormal accumulation of material with the ultrastructure of extrinsic fibers was present. The muscle layers of the most terminal region were unusually thickened and interrupted by fibrous septae. Immature neurons first appeared at E14, as in controls, but sometimes expressed an abnormal phenotype, such as that of sympathetic precursors with 50 um dense core granules. The development of the smooth muscle layer in the terminal bowel of transgenic fetuses was markedly retarded. These data suggest that congenital megacolon develops in the transgenic mouse because the terminal bowel contains small numbers of abnormal neurons, which fail to mediate the local reflexes upon which intestinal propulsion depends. The condition is not identical to that which appears in the mouse, although abnormalities of the developing smooth muscle and extracellular matrix are seen in both defects. Supported by NIH grants HD 17736, NS 15547, HD 18122.

448.8 EVIDENCE THAT MOVEMENT IN DROSOPHILA EMBRYOS IS NEUROGENIC AND MEDIATED BY GLUTAMATE. M.D.S. Anderson and H. Keshishian, Dept. of Biol. Yale University, New Haven, CT 06511.

It is known that the intersegmental neuromuscular junctions of Drosophila occur during stage 16 of embryogenesis and at this time the growth cones of these axons show positive immunostaining for glutamate (Johansen et al., Proc. Natl. Acad. Sci. USA. 1985.

In this study we are examining the development of the neuromuscular junctions of the bodywall muscles in Drosophila. We have begun using a glutamate-blocking spider venom fraction to examine the basis for these early muscle contractions. We have HPLC purified a component of the venom from the Weaver spider Araneus gemma that postynapticy blocks the glutamate response of the neuromuscular junction of Drosophila larval bodywall muscles. Glutamate-blocking activities of venom components were tested by physiological means using ommatophores of glumatate in the presence of various HPLC fractions. We found several with robust postynaptic effects as open channel blockers and have used one fraction to examine the development of neuromuscular contacts on the bodywall muscle fibers. By microinjection of the glutamate-blocking venom fraction into living embryos, we have been able to reversibly eliminate the muscle movements. Our results suggest that the embryonic muscle contractions are neurally evoked and likely due to glutamate release from the motoneurons. We are also examining similarities between our active fractions and other aragonins described in the literature (Adams et al., Bio. & Gene. Res. Comm. 148A785).
EFFECTS OF APOMORPHINE ON REGIONAL BRAIN NEUROTENSIN CONCENTRATIONS: IMPLICATIONS FOR DEVELOPMENT OF NOVEL ANTIPSYCHOTIC DRUGS. S. D. Nemeroff, C. D. Elite and B. Levant. A. Campbell and G. Blasucci. Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, NC and University of Pennsylvania School of Medicine, Philadelphia, PA.

Considerable data have revealed interactions between neurotensin (NT) and dopamine (DA) neurons. Centrally administered or locally infused pulses of NT and DA can be clinically efficacious antipsychotic drugs. We have suggested that antipsychotic-induced increases in NT in the n. accumbens (NA) are correlated with antipsychotic efficacy whereas NT increases in the caudate are associated with extrapyramidal side effect (EPS) liability. We sought to determine whether chronic treatment with (+)-NTA, an isomer of N-propionylnorapomorphine (NPA), produced selective inhibiting effects on mesolimbic DA systems alters regional brain NT concentrations. Adult male mice were treated chronically with haloperidol (3 mg/kg), S(+)-NPA (3 mg/kg tid), R(-)-NPA or vehicle for 10-15 days. RT was measured in 11 brain regions. Both haloperidol and S(+)-NPA, but not R(-)-NPA, produced significant increases in NT concentrations in the NA. In the caudate nucleus, haloperidol increased NT concentrations but S(+)-NPA and R(-)-NPA did not. These data provide further evidence for increases in NA NT concentrations by putative antipsychotic drugs. (Supported by NIMH MH-39415, NI-31967, MH-33006 and MH-41710)

SPECIFICITY OF ACTION OF CCK-4TETRAPERIDINE IN PANIC DISORDER. J. Bradwejn, D. Kozycz, C. Shriqui, G. Metealliar. Division of Psychopharmacology, St. Mary's Hospital, McGill University, Montreal, Quebec, H3T 1M5.

Cholecystokinin (CCK) is a peptide, fulfilling criteria for a neurotransmitter, found in high concentrations in the mammalian CNS. CCK-tetrapetidine (CCK-4) has been reported to induce panic attacks in panic-disordered patients (PD) (Bradwejn et al, Neuroscience 1988) and in healthy volunteers (HV) (de Montigny, C. Neuroscience, 1988). The purpose of this study was to determine the specificity of action of 30 ug of CCK-4 or saline on the ability of individuals to produce the release of NT in CSF. A total of 20 PD (mean age, 27.3 + 7.1 years; and 15 HV (5 F, 10 M), mean age: 27.3 + 7.1 years. Significant differences were present early in the course of the illness and cannot be explained by various confounding effects. Supported by MHO0423, MH42261, MH31154, MH/NS31862 and the Scottish Rite Foundation Research Foundation.

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ALTERNATIONS IN BILATERAL DISTRIBUTION OF BETA-RECEPTOR SUBTYPES IN SCHIZOPHRENICS AND SUICIDES. N. Lesch, R. Szeeman, R. Arismynsky, M. Cassoua, J. Kigouya, A. Windley and J. P. Joyce (UPON, J. Coustit) Departments of Psychiatry and Pharmacology, University Pennsylvania School of Medicine, Philadelphia, PA.

We have employed quantitative autoradiography to examine the distribution of Beta1 and Beta2 receptors in both hemispheres in several regions of postmortem brain tissue from: (i) schizophrenic patients that did not commit suicide, (ii) schizophrenic patients that did commit suicide, (iii) nonpsychotic suicide cases and (iv) nonpsychotic, nonviolent suicides. In hippocampus, thalamus and striatum an asymmetric distribution of Beta receptors was noted in the controls. The Beta2 subtype was higher in density in the right hemisphere of the hippocampus and thalamus, Beta1 was higher in the right striatum. In schizophrenic patients, this asymmetry was altered with an increase in Beta2 receptor density in the left hippocampus and a decrease in the right hippocampus. Similar results for Beta2 receptor density were observed in limbic cortical but not other cortical regions. In a number of nuclei of the amygdala there was a decrease in the left hemisphere in total Beta density. Patients who committed suicide with or without a diagnosis of schizophrenia showed a decrease in Beta1 receptor density in amygdala, the high density in the right hemisphere and decreased density in the thalamus. In contrast, schizophrenics who committed suicide showed different effects in high- and low-risk brain regions (like nonviolent schizophrenics). Funded by NIH NS19597, MH 43852 and MH43880.

CSF NEUROTENSIN CONCENTRATIONS IN PSYCHIATRY. G. Bissette, D. Harper, R. Kelley and C. D. Nemeroff. Department of Psychiatry and Psychology, Duke University Medical Center, Durham, NC and University of Cincinnati, Cincinnati, OH.

Neurotensin (NT) is an endogenous neuropeptide transmitter that shares many functional features with the antipsychotic drugs in laboratory animals. The CSF concentration of NT has been previously shown to be decreased in drug-free schizophrenic patients compared to controls. In the present study, we measured the CSF concentration of NT in chronic undifferentiated and chronic undifferentiated and schizoaffective (N = 9) subtypes of schizophrenia has been obtained. It is not possible as yet to determine whether these changes occur before, during or after the onset of schizophrenia, although our current evidence suggests that these differences are present early in the course of the illness and cannot be explained by various confounding effects. Supported by MHO0423, MH42261, MH31154, MH/NS31862 and the Scottish Rite Foundation.
CYTOARCHITECTURAL ABNORMALITIES OF THE ENTOURINAL CORTEX IN SCHIZOPHRENIA. S.E. Arnold*, B.T. Hyman and G.W. Van Hoesen (SPON: D.A. Moore). Department of Neurology and Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The cytoarchitecture of the entourinal cortex was examined in the brains of 6 patients with a diagnosis of schizophrenia and in 13 controls. All 6 schizophrenic brains showed cytoarchitectural abnormalities of the rostral and intermediate portions of the entourinal cortex. The abnormalities included aberrant nonsupervised imaginations of the anterior parahippocampal gyrus with disruption of cortical layers, heterotopic displacement of laminar-specific neurons and partial organization in superficial layers. Such changes would be consistent with disturbed development. The entourinal cortex is a pivotal point for neural systems that mediate hippocampal-cortical interactions. The disruption of these neural systems may have important neuropsychological sequelae, and contribute to the symptoms of schizophrenia. (Supported by: NS 14944.)

BIPOLAR AFFECTIVE VERSUS PURE MANIC DISORDER AFTER BRAIN LESIONS R.G. Robinson, S.E. Starkstein, P.Fedoroff*, M.L. Bentheimer*, H.S. Mayberg*, P.R. McHugh Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Although mania is a rare complication of brain injury, recent studies have found that lesion location, genetic predisposition, and subcortical brain atrophy are important correlates of this affective syndrome (Robinson et al., Am J Psychiátria 145:172, 1988). In the present study we compared patients who developed a bipolar affective disorder (i.e., mania and depression) after a brain lesion (n = 7), with patients who developed only mania (n = 12) (i.e., unipolar disorder). Although no significant group differences were found in demographic variables, the manic depressed group had significantly greater cognitive impairment than the mania only group (Mini-Mental State Exam Scores [mean ± SD]: 25.2 ± 4.7 and 28.6 ± 1.8, respectively, t = 2.21, p < 0.05). In addition, all of the bipolar patients had subcortical lesions (mainly involving the right head of the caudate and the right thalamus). In contrast, 10 of 12 patients with unipolar mania had cortical involvement (mainly right orbitofrontal and basotemporal cortices) (χ² Yates = 7.38, p < 0.01). These findings suggest that subcortical and cortical right hemisphere lesions may produce different neurochemical and/or remote metabolic brain changes which may underlie the production of either a bipolar disease or a unipolar mania.


We reported elevated D₂ dopamine receptor binding in drug naive schizophrenics (SCZ) (Science 234:1558, 1986), as shown by PET with [C-11] NMS NP. We have tested whether the following methodological issues contributed to our findings: [C-11] NMS NP metabolites measured by HPLC and calculated model input function correlations (N=36); (2) There were no differences in plasma protein binding of either [C-11] NMS NP in 21 patients, 8 SCZ (95.7 ± 4.0%),11 controls (95.8 ± 4.9%), and 8 [F-18] HAL in 10 SCZ (97.3 ± 0.2%), 6 normals (98.3 ± 0.2%), and 6 [F-18] HAL in 10 SCZ (97.2 ± 0.2%); (3) Biodistribution studies of [F-18] HAL in mouse brain performed in the presence of D₂ and sigma receptor antagonists gave results consistent with previous [F-18] HAL studies. No differences in bind (in [F-18] HAL partition coefficients in postmortem brain from 7 normals (3.1 ± 0.8 g tissue/ml plasma), 6 SCZ (3.1 ± 1.2), or 6 bipolars (2.9 ± 0.6), and values correlated closely with model assumptions. We conclude to first elevated mean D₂ in SCZ with these methodologic improvements in 19 drug naive SCZ (34.2 ± 19 pmol/g) and 7 psychotic bipolars (28.7 ± 12) compared with 19 controls (15.1 ± 6.6) and 7 non-psychotic bipolars (16.7 ± 8).


Several lines of evidence suggest a monoaminergic role in the pathophysiology of reduced prefrontal metabolism in schizophrenia. Recently, we observed that amphetamine, a direct dopaminergic agonist, increased prefrontal cortex (PFC) blood flow (XE-132 CBF) during the Wisconsin Card Sort (WCS), a PFC-driven cognitive task. To further explore the role of monoamines on cerebral function we conducted a double-blind placebo controlled cross-over study of the effects of 20mg/kg dextroamphetamine on cerebral blood flow (CBF) as determined by Xe-132 dynamic single photon emission computed tomography (SPECT) during performance of the WCS and a simple control task. Subjects included 9 patients with chronic schizophrenia who had been studied for six weeks on 4mg/kg haloperidol. Amphetamine produced a non-significant trend towards a diffuse reduction in rCBF that was independent of task. On placebo, no significant activation of CBF was seen during the WCS compared with the control task. With amphetamine, however, significant task specific activation of the left dorsolateral prefrontal cortex occurred (paired t = 4.75, p = 0.016). The results are further evidence that enhanced PFC monoaminergic activity may increase PFC metabolism and reverse "hypofrontality" in schizophrenia. In addition, on amphetamine, but not on placebo, a significant correlation (r = .73, p = .04) emerged between activation of PFC CBF and performance of the WCS task. These findings are consistent with animal models in which mesocortical dopamine activity modulates and enhances the signal to noise ratio of PFC activity.


We reported elevated D₂ dopamine receptor binding in drug naive schizophrenics (SCZ) (Science 234:1558, 1986), as shown by PET with [C-11] NMS NP. We have tested whether the following methodological issues contributed to our findings: [C-11] NMS NP metabolites measured by HPLC and calculated model input function correlations (N=36); (2) There were no differences in plasma protein binding of either [C-11] NMS NP in 21 patients, 8 SCZ (95.7 ± 4.0%),11 controls (95.8 ± 4.9%), and 8 [F-18] HAL in 10 SCZ (97.3 ± 0.2%), 6 normals (98.3 ± 0.2%), and 6 [F-18] HAL in 10 SCZ (97.2 ± 0.2%); (3) Biodistribution studies of [F-18] HAL in mouse brain performed in the presence of D₂ and sigma receptor antagonists gave results consistent with previous [F-18] HAL studies. No differences in bind (in [F-18] HAL partition coefficients in postmortem brain from 7 normals (3.1 ± 0.8 g tissue/ml plasma), 6 SCZ (3.1 ± 1.2), or 6 bipolars (2.9 ± 0.6), and values correlated closely with model assumptions. We conclude to first elevated mean D₂ in SCZ with these methodologic improvements in 19 drug naive SCZ (34.2 ± 19 pmol/g) and 7 psychotic bipolars (28.7 ± 12) compared with 19 controls (15.1 ± 6.6) and 7 non-psychotic bipolars (16.7 ± 8).

In order to characterize factors which regulate the spatial and temporal expression of the opiate neuropeptide met-enkephalin, the proenkephalin gene has been cloned in Xenopus laevis. A 2 kb genomic DNA-containing clone consisting of the third exon of the frog proenkephalin gene (obtained from G. Maron), Marone, G., Nature 309:251. 1984) was used to screen a Xenopus laevis genomic library. Novel NICHD restriction enzyme analysis showed that both positive clones in size 12 and 16 kb were isolated. These were shown to contain about 6 and 8 kb of 5' DNA sequences. Sequencing indicated that both clones contained the 3' end of the gene and the 5' end of the gene is not yet known.

Future studies will examine the effect of deleting the 5' upstream regulatory elements that have been identified on the initiation and patterning of proenkephalin gene expression.

ANGIOTENSIN II IS EXPRESSED IN SOLITARY VASOPRESSIN CELLS WITH A HETEROZYGOTIC PHENOTYPE IN HOMOZYGOTIC BRATTLEBRO RATS. P.W. van Leeuwen*, R. Ebbodam* and D. Felix**.

Netherlands Institute for Brain Research, Amsterdam, The Netherlands, **University of Berne, Switzerland.

In the homozygous Brattleboro rat (di/di) an altered vasopressin (VP) precursor is synthesized resulting in reduced hormone packaging. The expression of Angiotensin II (Ang II) immunoreactivity is also disturbed, and although dynorphin (DYN) is present in smaller vesicles than in heterozygous controls, it is not affected in di/di rats. Paradoxically, a small number of solitary hypothalamic neurons in di/di rats are expressing the wild-type VP precursor (i.e. VP, C-terminal VP-neurophysin and a glycopeptide). Previously we provided evidence that during life an increasing number of these post-mitotic neurons (up to 3% of the VP cells) undergo a switch to a genuine heterozygous phenotype. Thus, as di/di rats age, these hypothalamic neurons exhibit both the wild-type and the mutated VP precursors. Here we report the presence of Ang II in these heterozygous cells indicating that for the expression of Ang II a normal VP precursor is necessary. It is hypothesized that in the Golgi apparatus the various neuropeptides which are present in VP cells are packaged in granules in the following order: DYN first, followed by VP and Ang II.

REGULATED EXPRESSION OF α- and β-CGRP PROMOTERS IN TRANSFECTION CELL LINES. M.A. Kirschner* and S.G. Amar. Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale Univ. School of Medicine, 333 Cedar Street, New Haven CT, 06510.

The calcitonin/α-CGRP and β-CGRP genes code for two nearly identical neuropeptides that are expressed differentially and in discrete regions in the nervous system. Because they appear to be independently regulated and have highly divergent promoter sequences, they offer an excellent model system in which to explore the transcriptional regulation of the α-CGRP/calcitonin gene. Deletion mapping of the upstream regulatory elements that have been identified is being utilized to further delineate functional regions that mediate observed hormonal effects.

GENE EXPRESSION OF A NOVEL MAMMALIAN NEUROENDOCRINE POLYPEPTIDE (7B2) IN LOWER VERTEBRATES AND IN THE MARINE MOLLUSK PSEUDOCARDIUM. L. Sapunar* - M. MiksaK* - R. V. Chaitone* and V.F. Castellani. Clinical Research Institute of Montreal, Montréal, (Que), Canada, H2W 1B7. A highly conserved polypeptide called 7B2 has been isolated from porcine pituitary and is present in a DNA polypeptide is also widely distributed in the CNS but its biological role is not yet known. The mouse DNA sequence and partial amino acid analysis of this polypeptide has been determined (Mikaya et al., 1989). Northern and Southern blot analysis shows the expression of the 7B2 gene in other mammalian species (low, rat, mouse) and also in other vertebrates such as chicken and fish. Since the 7B2 gene appears to be highly conserved throughout evolution, we decided to study the presence of this polypeptide in the marine mollusk Apysia californica. By using a probe derived from the mouse 7B2 sequence, Northern blot hybridizations with highly stringent conditions were carried out. They revealed signals in the mRNA population of different organs such as ovotestis, hepatopancreas, stomach, kidney and CNS of Apysia. Similarly, signals were detected by Southern blot analysis in this invertebrate.

These preliminary results suggest that the 7B2 gene might be expressed in this system providing a useful tool for further investigation of the biological role of this polypeptide family.

CALCIUM CHANNEL MODULATION AND RESPONSE ELEMENTS OF THE RAT PROACTIN GENE. R.N. Dy* B.A. Bst*, R.A. Mauro*, and J.E. Engel* (SPON: R.A. Rottier). Dept. of Physiol. Biophys. University of Iowa, Iowa City, IA 52242 and Dept. of Pharmacology, Ohio State Univ., Columbus, OH 43210. In some endocrine cells, hormone synthesis may be regulated by Ca2+ entry through voltage-gated ion channels. We have used a hybridoma (DH5) Ca2+ channel modulator to explore the relationship between Ca2+ current and proactin (PRL) synthesis in pituitary cells, and to identify specific Ca2+ response elements within the 5' regions of the rat PRL gene. In patch clamp experiments, eumonitors of the DH5 Bk K644 expressed opposing effects on Ca2+ current through L-type Ca2+ channels in GH4C1 cells. The (+)-Bay K8644 enhanced the magnitude of Ca2+ currents 2-5 fold, while the (-) Bay K8644 blocked L channels. The eumonitors also produced opposite effects on PRL production by GH4C1 cells at concentrations in L-type Ca2+ channels in pituitary cells in culture. By using 500 fold concentrations of the (+)-Bay K644, Bk (20-72 hours) cells treated with 500 m (2-5)-Bay K644 typically produced 3-4 times more PRL than controls while the (-)-Bay inhibited PRL production by 30-65%. To assess the role of the 5' flanking sequences of the rat PRL gene in conferring Ca2+ and DH5 regulation of transcription, several fusion gene constructs containing 5' flanking sequences were coupled to the reporter gene bacterial chloramphenical transferase (CAT) and transfected into GH4C1 cells. Ca2+ stimulation actin expression in cells containing a construct which included 1.9 kilobase pairs of the 5' flanking sequence and the effect was enhanced by the (+)-Bay K644. Constructs containing either proximal (positions -292 to -38) or distal (positions -171 to -19) enhancers of the PRL gene also responded to Ca2+ and the agonist with enhanced expression of CAT. The additive response observed with the two enhancers (9 fold) was equal to that observed with the 1.9 kilobase construct alone. In conclusion, the eumonitors of Bay K644, by enhancing or blocking Ca2+ movement through L type channels in pituitary cells, produce opposing effects on PRL synthesis. These responses may be mediated by two separate Ca2+ response elements in the proximal and distal enhancer regions of the rat PRL gene.

FOSS AND JUN COOPERATE TO ACTIVATE TRANSCRIPTION OF THE HUMAN VIP GENE. M. Yehyav*, R. Goodman* and J.S. Fink. Div. of Molecular Medicine, New England Medical Center Hospitals, Boston, MA 02111 and Lab. of Molecular Neurobiology, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114 (SPON: A. Bockoms).

Transcription of the human VIP gene may be regulated by the integrity of an 17 bp enhancer element located near to its promoter. The sequence of the VIP second messenger-responsive element is similar to that of a TPA responsive element (TRE) and to other transcriptional activator protein A-1. The AP-1 family of enhancer binding proteins includes the protein products of the c-fos and c-jun genes. Fos and Jun proteins form a heterodimeric complex that interacts with AP-1 binding sites in several TREs. In this study we sought to determine (1) if Jun/AP-1 interacts with the VIP second messenger enhancer, and (2) if Jun and Fos cooperate to activate transcription of the VIP gene. Expression vectors containing human c-jun or human c-fos cDNAs were co-transfected into HepG2 cells with fusion genes containing 54 bp of the 5' flanking region of the VIP gene (containing the second messenger enhancer) linked to the reporter gene chloramphenical acetyltransferase (CAT). There was little or no transcription of the VIP-CAT gene by the c-jun or c-fos expression vectors when transfected alone with VIP-CAT. However, when c-jun and c-fos expression vectors were transfected together, there was 20-fold transactivation of the VIP-CAT gene. Similar results were obtained when the 17 bp VIP enhancer element was linked to a viral promoter. This suggests that Fos or Jun protein may be regulated by the second messenger via c-jun or c-fos protein.

In a DNase I footprinting assay purified AP-1, v-Jun or c-Jun protein protected a region of the VIP gene containing the second messenger enhancer. We conclude that transcription of the VIP gene by c-fos or c-jun may be regulated at multiple levels, other than transcriptional activation by Jun-Fos complexes with the VIP second messenger enhancer.
**450.7**

CYCLIC AMP- AND GLUCOCORTICOID-MEDIATED TRANSCRIPTIONAL REGULATION OF RAT PROENKEPHALIN GENE EXPRESSION IN C6 RAT GLIOMA CELLS. I. Ishii* and S.L. Sabol. Lab. of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892.

Glucocorticoids potentiate proenkephalin (pEnk) gene expression in several systems. Yoshikawa and Sabol (Proc. Natl. Acad. Sci. U.S.A. 85, 1988) showed that glucocorticoids such as dexamethasone (Dex) and adenylyl cyclase activators synergistically elevate the pEnk mRNA abundance in C6 rat glioma cells. We have investigated the mechanism of this effect by nuclear run-on transcription analyses and transfection assays. The pEnk gene transcription rate was not significantly altered by 1 μM Dex alone, transiently stimulated by 20 μM forskolin (up to 6-fold at 1 hr), and more persistently stimulated by Dex + forskolin (5-fold over 1-24 hr). Cyclheximide did not block these increases, indicating no requirement for ongoing protein synthesis. Increases in pEnk mRNA levels corresponded to increases in transcription rate increases. Dex did not alter the basal or forskolin-stimulated cAMP content of the cells and produced only a modest (30%) cAMP/histidine-sensitive elevation of cAMP-dependent protein kinase activity. These results suggest that cAMP and glucocorticoids cooperatively elevate endogenous pEnk gene transcription.

To search for functionally cooperative glucocorticoid and cAMP regulatory elements, rat pEnk genomic clones, isolated by Dr. H. Higuchi, were used to construct plasmids containing the chloramphenicol acetyltransferase (CAT) gene placed under the control of pEnk sequences from bases -2800 to +750 in a promoterless vector. In C6 cells transfected with these constructs, forskolin increased CAT activity 5-17-fold. Unexpectedly, Dex reduced basal and forskolin-stimulated CAT activity by up to 10% and 48%, respectively. These results suggest that the sequences tested contain, in addition to the cAMP regulatory element(s), a negative glucocorticoid regulatory element (GREG), while the expected positive or cooperative CRE may be outside this region.

**450.9**

EXPRESSION PATTERN OF NEUROPEPTIDE GENES IN TRANSGENIC MICE. D.A. Carter*, H.L. Ang* and D. Murphy*. (SPON: P.Hong). Institute of Molecular Cell Biology, National University of Singapore, Singapore 0511.

Neuropeptide gene regulation has been studied in transgenic mouse: bovine tran-gene expression is detected using species-specific mRNA and peptide probes. A 13.4 kb segment of bovine genomic DNA containing the vasopressin coding region and 7 kb of upstream sequence was expressed in the hypothalamus (low level) and ovary (high level) of one transgenic line. Observations throughout the estrous cycle indicate regulation of both transgene-derived mRNA, and VP in the ovary. Ovarian expression, which reflects the bovine pattern of endogenous expression, was also found in another Line containing a chimeric VP-oncogene construct (1.25 kb of VP upstream region linked to the SV40 large T coding sequence). In a third line (4.2 kb of bovine genomic DNA containing the oxytocin (OT) coding region and 0.6 kb of upstream sequence) OT expression was detected in the cerebellum, testis and lung. We have demonstrated expression and regulation of bovine neuropeptide transgenes in mice. Additional control sequences may be required to direct a high level of expression to hypothalamic neurons.

**450.11**


The four subunits of the nicotinic acetylcholine receptor are coordinately regulated during skeletal muscle development. To define DNA sequences involved in the transcriptional regulation of the α and β subunit genes, the 5′ flanking regions were fused to the chloramphenicol acetyltransferase (CAT) reporter gene and transfected into mouse myogenic cells. CAT activity was obtained in C2 myotubes, but not myoblasts when the -249 to -18 segment of DNA relative to the translation start site was used in both subclones used in conjunction with the SV40 early promoter. Further deletions resulted in decreased CAT activity, with a 40 bp fragment, 85% identical to the enhancer in the chick α subunit gene, giving 25% of the activity obtained with the full 5′ flanking long fragment. Relatively low CAT activity was observed in myotubes when β sequences from -890 to -1 relative to the translational start site were used, whereas when the -156 to -70 segment was used, higher CAT activity was observed in myotubes, with low activity in myoblasts. This higher activity was also obtained when a -156 to -70 fragment was fused to the SV40 promoter. Thus, there appear to be developmentally specific enhancers in both the α and β subunit genes, and a putative regulatory element in the β subunit gene.

We have recently cloned a cDNA for the type I, mineralocorticoid receptor (MR) from rat hippocampus (Patel et al., Neurosci. Abstr., V. 14, 1988). Comparison to human MR cDNA (Arriza et al., Science, 237:268, 1988) demonstrates uniform high homology throughout, except in the 5'-untranslated (5'-UT) region. RNAse protection of cDNA with hippocampal mRNA revealed 5'-UT sequence heterogeneity, suggestive of multiple mRNAs. To determine the source of this observation, a genomic fragment spanning a human-rodent genomic flanking region of the rat MR gene was isolated and partially characterized. Sequences were found corresponding to the 5'-UTs for both the beta (β) as well as the human (α) MR cDNAs, and appear to exist as distinct exons separated by approximately 2 kb, situated more than 4 kb upstream of an exon coding for the N-terminal domain. Both the α and β 5'-UT exons display consensuses for proper splicing to produce the observed mRNAs. Tag polymerase chain reaction is being used to determine if the homology of the human MR 5'-UT is expressed in mature rat MR mRNA.

It is not yet known what role alternate 5'-UT sequences play in expression of the MR. Quantitation of β and α forms does not reveal a clear tissue specific distribution, although there is a tendency for the β form to be expressed at higher levels in CNS tissues. Potential correlations may be found with development or in the stability/ translatability of the variant mRNAs.

PUTATIVE GENOMIC CLONES FOR HUMAN MAO A AND B. R. M. Denney and D. Pizzo. Dept. of Human Biological Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

We have isolated a genomic clone for the type I, mineralocorticoid receptor (C) from rat hippocampus (Patel et al., Neurosci. Abstr., V. 14, 1988). Comparison to human MR cDNA (Arriza et al., Science, 237:268, 1988) demonstrates uniform high homology throughout, except in the 5'-untranslated (5'-UT) region. RNAse protection of cDNA with hippocampal mRNA revealed 5'-UT sequence heterogeneity, suggestive of multiple mRNAs. To determine the source of this observation, a genomic fragment spanning a human-rodent genomic flanking region of the rat MR gene was isolated and partially characterized. Sequences were found corresponding to the 5'-UTs for both the beta (β) as well as the human (α) MR cDNAs, and appear to exist as distinct exons separated by approximately 2 kb, situated more than 4 kb upstream of an exon coding for the N-terminal domain. Both the α and β 5'-UT exons display consensuses for proper splicing to produce the observed mRNAs. Tag polymerase chain reaction is being used to determine if the homology of the human MR 5'-UT is expressed in mature rat MR mRNA.

It is not yet known what role alternate 5'-UT sequences play in expression of the MR. Quantitation of β and α forms does not reveal a clear tissue specific distribution, although there is a tendency for the β form to be expressed at higher levels in CNS tissues. Potential correlations may be found with development or in the stability/ translatability of the variant mRNAs.

ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING MURINE Tryptophan Hydroxylase. J. Slot and D. Goldman. Laboratory on Texas Medical Branch, Galveston, TX 77550.

A full-length tryptophan hydroxylase cDNA was isolated from P815 cells, a mouse mastocytoma cell line. This tryptophan hydroxylase cDNA we isolated from mouse mastocytoma differs from the 5'-UTs for both the beta (β) as well as the human (α) MR cDNAs, and appear to exist as distinct exons separated by approximately 2 kb, situated more than 4 kb upstream of an exon coding for the N-terminal domain. Both the α and β 5'-UT exons display consensuses for proper splicing to produce the observed mRNAs. Tag polymerase chain reaction is being used to determine if the homology of the human MR 5'-UT is expressed in mature rat MR mRNA.

It is not yet known what role alternate 5'-UT sequences play in expression of the MR. Quantitation of β and α forms does not reveal a clear tissue specific distribution, although there is a tendency for the β form to be expressed at higher levels in CNS tissues. Potential correlations may be found with development or in the stability/ translatability of the variant mRNAs.


The cellular regulation of RNA polymerase III (RPo III) mediated transcription is complex and not yet well understood. A number of factors, including sequence elements in the 5'-UT of the pre-mRNA and the presence of splice acceptors in the 5'-UT, may contribute to the regulation of RPo III activity. We have previously shown that the RPo III promoter sequence containing bases -151 to +27 is sufficient to drive expression of the Harvey ras oncogene when expressed in COS cells. We have now extended this analysis to the human RPo III gene that encodes the splice acceptor for the insulin gene. We have isolated a genomic clone coding for human MR-A and -B that contains all of the 5'-UT for both genes and extends into the first exon. This clone was used as a hybridization probe to isolate genomic clones from a rabbit reticulocyte lysate system using S-methionine to label the polypeptide.

SDF polyrmyosinigle gel electrophoresis of the translation products revealed a major band with a molecular weight of 66 kb. This band comigrated with authentic human MAO-A (provided by Walt Weyer). In addition, the translation product could be specifically immunoprecipitated with sheep antiserum prepared against MAO-A purified from human placenta (provided by John Pintar). Other experiments demonstrated that SDF-A or SDF-B could be targeted to rat liver mitochondria. Current studies are underway to determine which regions of the MAO-A and B are responsible for this targeting. (Supported by NIH grant NS21921)


Treatment of primary cultures of chromaffin cells with forskolin or TPA results in increased steady state levels of TH mRNA (Evinger et al., 1987). To determine if these agents act at the gene level, we isolated genomic clones containing the 5'UT and 1st exon of the human MAO gene containing bases -151 to +27 was fused to the bacterial CAT reporter and introduced into C6 glioma and Ltk cells for transient assay by the calcium phosphate technique. Expression of the TH-CAT constructs was readily observed in several nonneuronal cell lines indicating that elements controlling specific expression lie outside this region. The basal levels of expression differed in these two cell lines. While CAT activity measured in the C6 cells was 2 fold over promoterless plasmid controls, levels in Ltk cells were more than 10 fold above this background. However, when the transfectants were treated with activators of adenylate cyclase (forskolin 10 µM) and protein kinase C (TPA 10OnM), the levels under induced conditions were comparable for the two cell lines. In the presence of forskolin, CAT activity in transfected C6 cells was increased by 4-5 fold. TPA increased expression by 1.5-2 fold. These results suggest that the activation of second messenger systems acting through cAMP and protein kinase C leads to induction of TH transcription and that sequences residing within 151 bp of the transcription start site are sufficient to mediate these inductions. (Supported by MH24285-14 and grant from HoechstAG to MGH.)


Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia. GS plays a central role in nitrogen metabolism and ammonia detoxification in the central nervous system. Our interest in GS expression is due to its complex regulation by glucocorticoids, cAMP, insulin, and thyroid hormone, and its differential expression by astrocytes in the CNS.

We screened a rat cDNA library which was enriched in full length clones. A clone of 2.6 kb in length was selected through complementarity to a previously cloned GS DNA clone. The clone was not full length (Meawo et al., 1989). Sequence analysis of this clone shows it to be GS and to contain the 5' end of the message. This cDNA clone was used to isolate the gene from a rat genomic library constructed in Charon 4a. The 16 kb insert was subcloned as 4 Eco RI fragments. Sequence analysis has determined the first two exons and the 5' flanking region. The first exon extends 122 bp, from the transcriptional start site to upstream of the translation start site. The first intron is approximately 2 Kb in length and is followed by the second exon which continues for 176 bases.

Sequences with homology to the glucocorticoid and cAMP response elements, and the AP-2 transcriptional activator have been found in upstream flanking sequences. These regions may act as binding sites for the trans-acting factors responsible for transcriptional regulation of GS.
GLUTAMATE RECEPTORS MEDIATE IMMEDIATE-EARLY GENE EXPRESSION IN VIVO. B.A.Molar, Rode T. Current and IJ.Moghad. Deps. of Neurosciences and Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, nutley, NJ 07110.

Cellular immediate-early genes are defined by their similarities with regulatory genes of eukaryotic viruses, notably their rapid induction by extracellular stimuli, even in the absence of protein synthesis. The products of three such genes, c-fos, c-jun and jun-B, are components of transcription factors, and are expressed as nuclear third messenger molecules, coupling stimulation to long-term transcriptional responses. Here we describe the temporal pattern of expression of a number of cellular immediate-early genes in vivo (using Northern blot analyses; after activation of various classes of excitatory amino acid (EAA) receptors, BRAD, TACR, and trinitrotoluene (TNT), which activates the MAP kinase type of EAA receptor, causes a rapid and transient increase in c-fos and jun-B mRNA levels in brain. In contrast, c-jun rises and remains elevated at least 2 h after seizure. Kinase (KAI) stimulation results in a rapid, but much protracted, induction of c-fos and jun-B. However, c-jun is not strongly induced by this agent. LUNG: PTZ causes a minor, transient increase of c-fos and jun-B RNA in lung. However, c-jun is constitutively high, both before and following treatment. KAI, in contrast, produces a large increase in c-fos, which remains elevated for at least 2 h. c-jun transcripts are also rapidly induced by KAI and peak 45 min after administration, decreasing gradually thereafter. The response of jun-B is similar to c-fos. GUT: PTZ and KAI caused small increases in c-fos, c-jun and jun-B expression. However, basal mRNA levels of all three are higher in gut than either lung or brain. We establish here that activation of EAA receptors in vivo results in a tissue and ligand specific induction of three of the immediate-early genes.

Differential expression of ARPP-16 and ARPP-19, two closely related cAMP-regulated phosphoproteins. Anuko Horiuchi, Jean-Antoine Girault, Angu C. Naire and Paul Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

ARPP-16 and ARPP-19 are two cAMP-regulated phosphoproteins (Mr=16,000 and Mr=19,000, respectively). Sequence analysis of the cloned cDNAs and the purified protein indicates that these two proteins differ from each other by 16 additional amino acid residues on the NH2-terminus, which are present only in ARPP-19. Comparison of the base sequence of ARPP-16 and ARPP-19 cDNAs reveals extensive segments of total identity in the translated and untranslated regions. The structure of the genes encoding for these two proteins has been analysed by Southern hybridization of genomic DNA, using a variety of probes corresponding to the regions common to the two cDNAs and the regions specific to each of them. The regional, ontogenetic and phylogenetic distribution of ARPP-16 and ARPP-19 has been studied by Western blotting. ARPP-19 was found in the brains of all the vertebrate species studied, and at various levels in all the tissues of adult rat. ARPP-19 was also present at high levels in several malignant cell lines and in normal embryonic tissues where it decreased during development. In contrast ARPP-16 was detected only in some specific neurons of the dopamine-innervated regions of the cerebral cortex and the basal ganglia, which are known to possess the D1 dopamine receptor. ARPP-16 was phylogenetically recent, being found only in birds and mammals and appeared late during ontogenesis, during the postnatal period. These observations suggest that the regulation of the expression of ARPP-16 and ARPP-19 is very different. ARPP-19 is expressed in all cell types especially when they are not fully differentiated, while ARPP-16 appears to be expressed only in neurons which possess the dopamine D1 receptor.


Oligonucleotides were synthesized to hybridize to either the inducible HSP70 mRNA, the constitutively expressed cognate HSC70 mRNA, or the glucose-regulated protein, GRP78 mRNA. HSP70 mRNA encoding GRP78 (3.05 kb), and HSP70 (2.55 kb) were detected in all tissues. No HSP70 mRNA was detected in control tissues. After heat shock or amphetamine-induced hyperthermia, GRP78 mRNAs encoding GRP78 (3.05 kb), and HSP70 (2.55 kb) were induced in all tissues. No HSP70 mRNA was detected in control tissues. After heat shock or amphetamine-induced hyperthermia, GRP78 mRNAs encoding GRP78 (3.05 kb), and HSP70 (2.55 kb) were induced in all tissues. We have synthesized oligonucleotide probes that specifically recognize the stress-induced (HSP70) or constitutively expressed (HSC70) heat shock mRNAs. The two mRNAs are quantitated in in situ hybridization in different cell types of rat cerebellum relative to total poly(A) and 18S mRNA. No HSP70 mRNAs were found in control rat cerebellum. GRP78 mRNAs and HSP70 mRNAs were found in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells. After amphetamine-induced hyperthermia, HSP70 mRNA was detectable in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells. After amphetamine-induced hyperthermia, HSP70 mRNA was detectable in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells. After amphetamine-induced hyperthermia, HSP70 mRNA was detectable in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells. After amphetamine-induced hyperthermia, HSP70 mRNA was detectable in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells.
451.3 REGULATION OF TRANSLATION BY STEROIDS: MYELIN PROTEIN mRNA.
J. M. Verdi*, S. W. Grant*, AND A. T. Campagnoni. (SPON: C. Clemente)
UCLA, Medical School, 760 Westwood Plaza, Los Angeles, CA 90024.

The translation of several myelin protein mRNA species were
examined in reticulocyte lysates and myelin basic protein (MBP) mRNAs were
found to translate less efficiently than other mRNAs tested. Among
the transcripts of MBP, four different isoforms of MBP (M.30k, MBP-
18kDa MBP, MBP-15kDa MBP, and MBP-13kDa MBP) were
translated more efficiently than the others. This indicates that
coding regions in the mRNA, in addition to the 3' untranslated regions, can
influence the efficiency with which mRNAs are translated. The role of the
7me-guanosine cap in the translation of myelin protein mRNAs was also
examined. MBP mRNA was translated more efficiently when it was capped.
In contrast, MBP mRNA translated 1.6x better when they were uncapped.

The functional role of these steroid-induced changes in translation is being
investigated. The functional role of these steroid-induced changes in translation
is being investigated.

451.4 HEAT SHOCK RESPONSE IN GEBRILÞER AFTER ISCHEMIA - IN SITU
HYBRIDIZATION ANALYSIS. T. S. Nawark, Jr. Lab. of Neuropathology and
Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892.

The distribution and induction time course of RNA encoding the 70
kDa stress/heat shock protein (hsp70) was determined in postischemic
gerbil brain by in situ hybridization with a 35S-labeled oligonucleotide
selective for inductive species of the hsp70 family, and compared with
that of immunoreactive hsp70 detected with antisera having similar selectivity.
In control animals hsp70 hybridization and immunoreactivity were detected only in
ependymal cells. Following 5 min bilateral carotid artery occlusion, hsp70 RNA was evident
in denate granule cells within 3 h, with prominent induction in all
hippocampal pyramidal cell fields by 6 h recirculation. Within 24 h
the signal was greatly reduced in those neuron populations which
previously showed a robust hsp70 immunoreactivity. The hsp70 RNA
sequences remained elevated through at least 48 h in CA1 neurons
which fail to express the protein. No hsp70 hybridization remained in
the CA1 region following the loss of these neurons by 4 d. CA1 neurons
vulnerable to ischemic injury thus demonstrate a robust and even
prolonged heat shock response at the transcriptional level under
conditions in which profound deficits in translational activity may
preclude accumulation of immunoreactive protein. In CA3 neurons
there was a notable lag between the induction of hsp70 mRNA
transcripts and the appearance of a substantial increase in hsp70
immunoreactivity. In CA1 neurons there was a substantial increase in hsp70
immunoreactivity. In CA1 neurons there was a substantial increase in hsp70
immunoreactivity.

Four forms of 1B426B mRNAs are generated from 2 genes by alternate transcriptional initiation and subsequent 5' end splicing. The mRNAs are most abundant in cortex and hippocampus, but are present at lower levels in other brain regions. Some forms are found in putative adrenal (adrenal), spleen, thymus and ovaries, but none is found in any of several other tissues. In situ hybridization shows enrichment in dentate granule cells and polymorphic neurons, and pyramidal neurons of the hippocampus (CA4-CA3-CA1) and neocortical layers 3,5 and 6. 1B426B mRNAs encode 4 distinct, apparently secretory and/or membrane proteins of 125, 153, 457 and 485 amino acids that differ from each other only at N and/or C termini. Antisera to synthetic peptide fragments of the common region detect four proteins in neocortical layers 3,5 and 6. 1B426B mRNAs encode proteins of 125, 153, 457 and 485 amino acids that differ from each other only at N and/or C termini. Antisera to synthetic peptide fragments of the common region detect four proteins in neocortical layers 3,5 and 6. 1B426B mRNAs encode proteins of 125, 153, 457 and 485 amino acids that differ from each other only at N and/or C termini. Antisera to synthetic peptide fragments of the common region detect four proteins in neocortical layers 3,5 and 6.
A novel in vitro cDNA amplification method: Linear amplification of rat cerebellar cDNA.

**451.1**


Trypsin-inhibitor (TI) is a potent releaser of endogenous CCK in adult rats (Liddle et al., *Gastroenterol.*, 87:524, 1984). Assuming a similar effect in the young rat, TI was used to test the hypothesis that endogenous CCK would reduce the intake of milk diet in neonatal rats. The rats were placed on a tube soaked with commercially available 'Half and Half'. TI significantly reduced the percent body weight gain in two separate replications: mean = 1.28% vs 0.1% and 1.29% vs 0.34% for the saline-TI comparisons in the two replications (p<0.05; N=8 rats per infusion group in each replication). These results suggest, but do not prove, that TI decreased intake by releasing CCK from the small intestine.

**452.4**


Raclopride, a D-2 dopaminergic antagonist, decreases the sham feeding of sucrose in adult rats (10 mg/kg, ip, Schneider et al., 1988). To determine the ontogeny of this effect, pups were removed from the litter on postnatal days 7, 14, and 21. Some pups were equipped with an anterior, sublingual catheter (Hall, 1979). Four to 6 h later, the intake of 10% sucrose was measured in one of two 2-h tests (Table). Raclopride (10 mg/kg, ip) was injected 15 min prior to an intake test. Each pup was tested only once. Raclopride was significantly more potent for decreasing intake in the independent ingestion test than in the introrctal intake test of Day 7 (Table).
452.5


We have recently refuted the suggestion that a neurotransmitter systemic of corticotropin releasing factor (CRF) is central to the suppression of food intake (FI) observed in protein energy malnutrition (PEM). Brain histamine and CRF as well as transporters for these neurotransmitters are upregulated by PEM. The present experiment (18 months old male rats) showed that systemic CRF, but not histamine, suppression of FI is partially ameliorated by administration of CRF desoctapeptide.

To further our hypothesis, we evaluated the effect of PEM on several parameters of satiety-related function. Additionally, we assessed the effect of electrolytically ablating the periventricular nucleus of the hypothalamus (PVN) in FI of PEM rats. The results (in all studies) show that male rats assigned to treatment groups and studies were conducted according to a randomized block design. Control diets contained 25% casein. Body weight parameters of pituitary-adrenal function. Additionally, we assessed the effect of PEM on several parameters of satiety-related function. Additionally, we assessed the effect of electrolytically ablating the periventricular nucleus of the hypothalamus (PVN) in FI of PEM rats. The results show that male rats assigned to treatment groups and studies were conducted according to a randomized block design. Control diets contained 25% casein. Body weight was measured daily and food intake was measured daily. The results show that male rats assigned to treatment groups and studies were conducted according to a randomized block design. Control diets contained 25% casein. Body weight was measured daily and food intake was measured daily.

E.G. Gisel. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, H3G 1Y5.

Supported by NHRDP # 6605-240.

452.6


From data collected using rats as a model, a conditioned taste aversion as a measure of satiety, we proposed a neuroendocrinological model of anorexia nervosa. We suspected that fetal DES could be involved in anorexia nervosa. Suggest that fetal DES could be involved in anorexia nervosa. Our study described the effect of food textures on tongue movement during anticipation, chewing, and swallowing in children six months to two years of age. Tongue mobility changes rapidly in the human infant during weaning. The anterior-posterior movements of sucking which are activated in concert with the mandible, hyoid and lower lip give way to more independent movements in a lateral direction. These independent tongue movements between the teeth are present during the feeding stages, suggesting tongue’s role as a neurotransmitter within these nuclei. The VMH and LH were dissected and assayed for free amino acid and somatostatin concentration in response to meal feeding regimen in 16- and 3 month old rats. Frozen transverse sections (20 µm) were incubated with 10nM [3H] MAZ in 50mM Tris/5mM KC1, pH 7.9, at 4°C for 30 min. Non-specific binding was assayed with 1mM MAZ. The distribution of these sites was different from that of the high affinity, sodium-dependent binding of [3H] MAZ to catecholamine receptors which were highly localized in the striatum. Areas of high specific [3H] MAZ binding (>3.0pmol/mg protein; 53-69% of total binding) were found in the dorsal raphe n., locus coeruleus, med. eminence, olf. tub., pineal gland. Moderate binding (1.6-3.0pmol/mg) was found in the paraventricular n. and the med. eminence. Low levels (<1.0pmol/mg) were found in the substantia nigra. These areas showed the highest levels of [3H] MAZ binding. 452.10


This presentation reports 1) the results of adult conditioned taste aversion establishment using 3 estrogen doses in post-menopausal DES & lactostatin in unexposed male & female rats (N= 6 rats/group); 2) an analysis of data from 1711 DES fataley exposed & 519 unexposed human females whose weights are 19-31000 cases of unexplained low body weight (Weight<80% of expected for age, sex, height) in the exposed group compared to 3/10000 cases in the unexposed group: a 5.72 to 1 ratio
452.11

Paradigms for studying activity-based anorexia (ABA) and activity-stress gastric ulcers (ASUs) involve concurrent introduction of restricted feeding and opportunity for exercise. While ABA is related to the simultaneous introduction of diet and exercise, it is possible that ASUs contribute to the phenomenon. Also, there is evidence suggesting that susceptibility to both ABA and ASUs may be gender specific. This study tests the hypothesis that ABA is unrelated to the formation of ASUs: In body weight matched, pubescent male and female rats, a 25% weight-loss criterion was used to define ABA. The rats (41-43 days old) were fed for 30 min. and allowed 22.5 hours access to an activity wheel. Although female rats ate less and ran more than males, both males and females reached the weight-loss criterion in the same number (6.13) of days. Sacrificed at 75% of their original body weight, no animal showed gross evidence of lesions or ulcers. We conclude that at this weight loss criterion ABA is not gender specific and is not attributable to the presence of gastric lesions or ulcers.

452.12
AMPHETAMINE AND PHENYLPROPANOLAMINE: EFFECTS ON FEEDING IN THE RAT. P.J. Wellman, R. Cockroft* and S. Keller*. Dept. of Psychology/ Texas A&M University, College Station, TX 77843.

Memorial Sloan-Kettering Cancer Center, New York, New York 10021; Department of Psychology, Texas A&M University, College Station, TX 77843; Department of Psychology, West Virginia University, Charleston, West Virginia 25304.

Paradigms for studying activity-based anorexia (ABA) and activity-stress gastric ulcers (ASUs) involve concurrent introduction of restricted feeding and opportunity for exercise. While ABA is related to the simultaneous introduction of diet and exercise, it is possible that ASUs contribute to the phenomenon. Also, there is evidence suggesting that susceptibility to both ABA and ASUs may be gender specific. This study tests the hypothesis that ABA is unrelated to the formation of ASUs: In body weight matched, pubescent male and female rats, a 25% weight-loss criterion was used to define ABA. The rats (41-43 days old) were fed for 30 min. and allowed 22.5 hours access to an activity wheel. Although female rats ate less and ran more than males, both males and females reached the weight-loss criterion in the same number (6.13) of days. Sacrificed at 75% of their original body weight, no animal showed gross evidence of lesions or ulcers. We conclude that at this weight loss criterion ABA is not gender specific and is not attributable to the presence of gastric lesions or ulcers.

452.13
PERIFORNICAL HYPOTHALAMIC MICROINJECTIONS OF AMPHETAMINE AND PHENYLPROPANOLAMINE: EFFECTS ON FEEDING IN THE RAT. J.P. Hallman, R. Cockroft and S. Keller*. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Amphetamine anorexia results from activation of dopaminergic and/or beta-adrenergic cells within the perifornical hypothalamus (PFH) but the locus of anorexia induced by the amphetamine analogue phenylpropanolamine (PPA) is yet unidentified. In the present experiment, adult male rats were allowed to consume a palatable sweetened diet with a 0.3 M sucrose solution prior to feeding. Prior to the feeding trial with vehicle trials interspersed between drug trials. Amphetamine significantly lengthened latency to feed and suppressed feeding. PPA was without significant effect on feeding at any dose. These data support the contention that although amphetamine and PPA are structurally similar, the mechanisms by which these drugs act on feeding are independent.

452.14
CIRCADIAN FLUCTUATIONS IN TASTE PREFERENCES IN RATS. D. Dracos and F.W. Flynn. Dept. of Psychology and Neuroscience Program, Univ. of Wyoming, Laramie, WY 82071.

Day/night fluctuations in internal state are thought to mediate the periodicity in ingestive behavior in animals. Since experimental manipulations of metabolism and water/electrolyte balance modify taste processing, the effects of circadian changes in internal state on taste preference were measured. Rats were entrained to a 12 h light/dark cycle. Two bottles, one containing water and the other the taste stimulus (sucrose - 0.01 M, 0.03 M, 0.3 M, 1.0 M; quinine HCl - 0.00003 M, 0.0003 M, 0.003 M, 0.03 M; HCI - 0.003 M, 0.003 M, 0.03 M, 0.3 M), were presented at the onset of either the light cycle (light=12 h) or dark cycle (dark=12 h). Intake was measured 12 h later and preferene computed. During the dark period, the preference for 0.03 M sucrose (87 ± 2%) and 0.1 M NaCl (65 ± 4%) were significantly greater than that reported during the light period (55 ± 6% and 66 ± 6%, respectively), p<.05. Also, during the dark phase, the rats' preference for NaCl concentrations was consistently lower than that for the light phase, p<.05. There were no significant group differences in HCl preference. The results demonstrate that the rats' preferences for different tastes are influenced by the light cycle. (Supported by NIH grant RO1 NS24879 to F.W.F.)
or decrease the DA single unit activity, and the sigma receptor that sigma receptor agonists [(+)-3-PPP, (+)-pentazocine, DTG] feedback loop which is thought to be responsible for the DA unit activity, control rats trained to exert between 10 and 15 g of paw pressure for 1 second in order to procure a water reinforcement) required an average of 4 band reinforcers/reinforcer, whereas lesioned rats were significantly impaired, requiring an average of 8 band reinforcements. This indicates that, although lesioned rats obtained a comparable number of reinforcers as the controls, they performed less efficiently. In addition, the drug challenge was observed that lesioned rats were sensitized to the acute effects of apomorphine on the rotated task and were more sensitive to the acute effects of oxotremorine on the force lever task as measured by estimated ED$_{50}$ doses. These observations are evaluated in light of the magnitude of the MPP+-induced neuronal chemical lesion of the striatum and the degree of impairment observed.

Some emerging data are suggesting functions for pathways from the midbrain with monoamine (norepinephrine, dopamine, and serotonin) neurotransmitters. One pathway, involving the basal ganglia, appears to be important in the selection of motor plans. Another pathway, involving the limbic system, appears to be involved in regulating that plans once selected are actually executed. Different control functions are suggested each of the transmitters in pattern matching. The prefrontal area of the cerebral cortex appears to be engaged in the integration of these two circuits. Such characteristic motor disorders as Parkinson’s disease, La Tourette’s hyperkinetic-disruptive disorder (which has both motor and ideational components) can be understood as breakdowns in one or another circuit.


Autodragnostic studies which have demonstrated the presence of sigma receptors on substantia nigra dopamine (DA) neurons have provided the speculation that sigma receptors may have a modulatory role in DA neurotransmission. Support for this hypothesis comes from in vivo studies demonstrating that sigma receptor agonists [(+)-3-PPP, (+)-pentazocine, DTG] decrease the DA single unit activity, and the sigma receptor antagonist BMY-14802 decreases DA single unit activity. This study evaluated the interactions between haloperidol and the sigma antagonist BMY-14802. Haloperidol, up to 1 mg/kg, i.v., does not produce its typical increase of substantia nigra DA unit activity when pretreated with 1 mg/kg BMY-14802. This suggests a role of the sigma receptor in the nigro-striatal feedback loop which is thought to be responsible for the DA unit rate increases produced by haloperidol. The BMY-14802 dose response curve for producing DA unit rate increases was minimally affected following pretreatment with haloperidol. Since BMY-14802 does not bind to the sigma receptor sites, we hypothesize that the sigma receptor-induced rate increases are mediated at the DA cell body.
CLOZAPINE IN FREELY MOVING RATS. Ľ.L. Haracz. J.T. Tschanz*. I.M. Psychology, Indiana Univ., Bloomington, IN 47405. White*. Z.R. Wang*. D.W. Miller*, and G.V. Rebec. Prog. Neural Science, Dept. accumbens in rats subjected to simultaneous videotaping of behavior and recording of single-unit activity. To date, 17 neurons exhibited firing rates of 1.92±0.78 spikes/sec during quiet rest, 14 of which were activated during motor activity, such as locomotion (n=9) or head movements (n=5). Many neurons altered their activity in relation to the probing of specific body regions. D-AMP (1.0 mg/kg) was administered 30 min before either HAL (1.0 mg/kg) or CLZ (10.0 mg/kg). AMP- induced stereotyped behaviors developed in the rats, which increased (n=15) or decreased (n=4) in neuronal activity, with increases predominating among motor-related neurons. HAL inhibited behavior while suppressing the activity of all 13 cells tested. Compared to lateral neostriatal neurons (Haracz et al., Soc. Neurosci. Abstr. 14:664, 1988), the disparity in behavioral effects of these drugs. (Supported by USPHS grant DA 02451.)

BLO C K ER INTO TH E V E N T R A L  T E G M E N T A R E A  OF RATS. AND  CHRONIC M ICROINJECTIONS OF A K +-C H A N N E L


Apamin is an octadecapeptide which has been reported to block small conductance voltage-activated K+-channels. The present report describes behavioral and neurochemical effects of an acute and protracted administration of apamin into the VTA led to a dose-related (1.0-3.0 pmol/side) increase in motor activity. HPLC analysis of postmortem brain tissue obtained 30 min post-injection revealed a dose related increase in DA metabolism in both the VTA and the nucleus accumbens, an anterior terminal field of the VTA. Daily microinjections of apamin into the VTA appeared to initially lead to tolerance following an augmentation of motor activity one week after protracted treatment. These results support a role for SK channels in modulating DA stimulated motor activity.

AN A NEW COMPUTER PROGRAM FOR THE CONTROL OF IN VIVO BRAIN SELF STIMULATION: APPLICATION TO THE EFFECTS OF A NEW CLASS OF CENTRAL STIMULANTS. T. Kling-Petersen* and K. Svensson* (spon. J. Engel). Dept. of Pharmacology, Univ. of Göteborg, PO Box 33031, S-400 33 Göteborg, Sweden.

The in vivo brain self stimulation paradigm has been used for many years in investigating the biochemical rates of reward or placebo reinforcement. In general, central stimulants acting by the catecholamine neurotransmitters (e.g. dopamine or serotonin) or other neurotransmitter morphine) facilitate self-stimulation. Recently, our group presented a new class of central stimulants: "preferential dopamine autoreceptor antagonists" (K. Svensson et al., Naunyn-Schmiedebergs Arch. Pharmac., 334, 234-41, 1986). This class of compounds (exemplified by (+)-AJ76) produces mild stimulation over a wide dose-range. The degree of stimulation is however limited by the postsynaptic dopamine receptor blockade induced by large doses of the drug. It would be of great interest to have these types of compounds characterized in the self-stimulation paradigm.

A fully automated self stimulation method is currently being set up. This system will be based on standard sound-proof lever pressing boxes and physiological stimulatators. The equipment will be controlled by an Apple Macintosh™ IIx computer with 8 Mb primary memory and the National Instruments Lab VIEW program with a 12 bit analog/digital converting board. Our main objectives when setting up the method are: possibility to record at least 4 animals simultaneously, ability to vary the response rate of the different animals independently, to get total calculations at specified time intervals and continuously record on disc.

The computer program will be presented and, if available, preliminary data on the new type of central stimulants as well as reference compounds.


(+)-AJ 76 was reported by Svensson et al. (1986) to have a profile of activity suggestive of selective dopamine autoreceptor antagonism. This study compared (+)-AJ 76 to the specific D2 antagonist, sulpiride, in dopamine-related activities. Spontaneous locomotor activity of rats was significantly increased at low doses and decreased at high doses by both compounds. (+)-AJ 76 is similar in potency to sulpiride. The locomotor depressant effect from a low dose (0.03 mg/kg) of apomorphine was partially reversed by both (+)-AJ 76 and sulpiride. Both compounds also antagonized yawning produced in rats by the selective D2 agonist, quinpirole (0.06 mg/kg). (+)-AJ 76 is slightly more potent than sulpiride. Hypospadias effect produced by quinpirole (1 mg/kg) in mice is completely antagonized by the pretreatment with either (+)-AJ 76 or sulpiride. On the other hand, stereotypic licking and chewing produced by a high dose (3 mg/kg) of apomorphine in rats was not antagonized by either compound even at a high dose of 100 mg/kg. The emetic effect of apomorphine (0.1 mg/kg) in dogs was effectively blocked by sulpiride (ED50=0.2 mg/kg). (+)-AJ 76 did not antagonize apomorphine in this test at doses up to 3 mg/kg. We conclude that (+)-AJ 76 is similar to sulpiride as a selective D2 antagonist. Both compounds preferentially antagonize dopamine at the autoreceptors.


The striatum is functionally heterogeneous in that anatomically discrete areas of the striatum control different behaviors. Recently, Pisa et al. and Schwan (Brain Res. 102:429-440, 1976) using ibotenate-induced lesions, and Kelley et al. (Psychopharmac., 55:236-259, 1986) using microinjections of d-amphetamine, have shown that the ventral lateral striatum is involved in mediation of tongue and mouth movements. We report the effects of placing fetal nigral dopamine tissue grafts in the striatum on the behavior of rats licking drops of water. The rats were first given unilateral 6-hydroxydopamine lesions and tested for amphetamine induced rotation to assure they had greater than 95% depletion. Fetal nigral transplants from 12 to 15 days old rat fetuses were implanted into the ventral lateral (5mm) and dorsal medial (6mm) striatum of the lesioned side. A control group (n=5) was lesioned but did not receive fetal grafts. Nine months after transplantation, the rats were exposed to a schedule of periodic water presentation in which they received a .025 ml drop of water every 30 sec over a 30 min period. There were no group differences in baseline licking behavior. When given 0.1 mg/kg d-amphetamine rats with lateral but not medial transplants licked more than controls.

REAGTAINED ALTERATIONS IN INTRACRANIAL SELF-STIMULATION FROM THE NUCLEUS ACCUMBENS OF BALB/cByJ, C57BL/6J and DBA/2J MICE: EFFECTS OF DIAZEPAM. G. MacNeil, N. Gabora*, S. Collins* and R. Zacharko, Dept. Psychology, Carleton University, Ottawa, CANADA.

Uncontrollable stressors induce disturbances in responding for electrical brain stimulation (ICSS) from both mesolimbic and mesocortical sites. Investigations in this laboratory focused on the effects of uncontrollable footshock on ICSS in both inbred and non-inbred mouse strains. In the present report the effects of restraint were evaluated on ICSS from the nucleus accumbens in the BALB/cByJ, C57BL/6J and DBA/2J strains. In protracted immobilization was associated with deficits in ICSS. When BALB/cByJ and DBA/2J mice, such effects were absent in the C57BL/6J strain. These ICSS deficits were restricted to the anterior plane of the accumbens and prophylactic administration of diazepam produced an attenuation but not a complete reversal of the ICSS alterations. It is suggested that the anatomical/neurochemical integrity of the mesolimbic system in a rostral-caudal plane underlies the disparity in behavioral effects observed.
453.13 STRAIN DIFFERENCES IN RESPONDING FOR SELF STIMULATION FROM THE VENTRAL TEGMENTUM FOLLOWING ACUTE AND CHRONIC SHOCK. M. Kassab* and R.M. Zacharko, Dept. Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Acute and chronic footshock influenced self stimulation responding from the lateral aspects of the ventral tegmentum. However, the extent of the disruption induced by acute shock is dependent on the course of the contamination associated with a chronic stressor varied across strains of mice. In C57BL/6J and CD-1 mice a single shock session provoked a persistent disruption of ICSS performance. Responding recovered to baseline rates within 5 shock sessions, and in C57BL/6J mice responding exceeded baseline rates after 10 sessions. In contrast, in DBA/2J mice the decline of responding was not evident until 10 shock sessions were applied, and recovery of responding was evident after 15 sessions. Finally, in BALB/cByJ mice increased rates of responding were evident regardless of the number of stressor sessions applied. It is suggested that analyses of stressor related behavioral disturbances should consider genetic differences in the impact of acute stressors and in the adaptation associated with chronic stressor regimens.

453.14 Ontogeny of dopamine-mediated behavior and D1 receptors in three rat strains. Frances Petracca*, Jaime Diaz, and Alan S. Uts, Departments of Psychiatry and Psychology, University of Washington, Seattle, WA 98195

The F344 rat is a proposed model of Attention Deficit-Hyperactivity Disorder (ADHD) because of its increased locomotor behavior compared to the Wistar rat. Our preliminary observations regarding the ontogeny of this behavior are reported here. D-1 receptors, which mature functionally before D-2 receptors, were also examined. Ontogeny of developmental milestones, reflexes, and open field behavior were studied in pups from these two strains and the Sprague-Dawley (SD) strain. SDS-PAGE autoradiography revealed anatomical localization to cortical regions in Day 4 pups which was eliminated by Day 21. Similarly, locomotor behavior was increased from Day 4 to 21. Significant strain-related differences in indices of maturation and behavior are shown below:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Eye opening (day)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344</td>
<td>13.3+/-2.5</td>
<td>32.5+/-3.1</td>
</tr>
<tr>
<td>SD</td>
<td>13.2+/-1.3</td>
<td>36.5+/-2.6</td>
</tr>
<tr>
<td>BUF</td>
<td>12.5+/-0.9</td>
<td>38.4+/-5.7</td>
</tr>
</tbody>
</table>

These studies may support the use of the F344 rat as a model of ADHD.

NEUROETHOLOGY III

454.1 The One-Dimensional Protein Profiles of Gymnotiform and Mormyrid Electric Organ Homologs are Similar. L. Patergnon* and M. D. Fahon, The University of Texas at Austin, Dept. of Zoology, Austin, Texas 78712. The two orders of electric fish, the gymnotiforms of South America, and the mormyrids of Africa, possess distinct phylogenetic histories, distinct electric organ discharge patterns, and are derived from different branches of the electric fish evolution. However, both groups have electrocyte cells which are embryologically derived from muscle cells. To determine what differences might exist in the proteins present in the Mormyrid and gymnotiform electric organ, and to compare the protein composition of both electrocyte cells and skeletal muscle cells from various species, one-dimensional gel electrophoresis was used.

Electric organ tissue from six species and muscle tissue from three species of electric fish were analyzed. Electric organs from four gymnotiform species (Apnteronotus albifrons, Apteronotus leptorhynchus, Eigenmannia virescens, and Eigenmannia hambaudi) and two mormyrid species (Gnathonemus petersii and Brachycephalus ritteri) were compared. Muscle tissue from one gymnotiform (Apteronotus albifrons) and one mormyrid species (Gnathonemus petersii) were compared. The relationships of these data were calculated using a genetic distance coefficient of 1-0.471. The results show that the gymnotiforms and mormyrids have a common ancestor, but that the gymnotiforms have diverged from the mormyrids. Further, the results suggest that the gymnotiforms are more closely related to the mormyrids than to the other gymnotiform species.

454.2 Synaptic Plasticity in the Cerebellar Parallel Fiber Projection to the Electrosensory Lateral Line Lobe of Gymnotiform Fish. R.W. Turner* and L. Waser (SPON: W. Hendelman) Dept. of Anatomy, University of Ottawa, Ottawa, Ontario, Canada K1H BM5

Pyramidal cells (PC) of the electrosensory lateral line lobe (ELL) of the weakly electric fish, Aequorea spec., have been shown to be depolarized by the release of a neurotransmitter from the axon terminal of a signal neuron. The role of excitatory amino acid receptors in synaptic plasticity are under investigation.

In vivo electrophysiological recordings from ELL PC apical dendrites in the ELL molecular layer receive a glutamatergic parallel fiber input from granule cells of the caudal cerebellar lobe. This feedback projection to the ELL has been shown to regulate PC temporal and spatial receptive field properties (Bastian, '86). As the molecular layer exhibits high densities of NMDA and Kainate receptors (Maior & Monoghan, '89) frequently involved in plasticity of synaptic transmission, we used an in vivo slice preparation to examine PC responsiveness to parallel fiber stimulation.

Stimulation of the ELL molecular layer evoked a fast positive-negative and a slow negative-going field potential in the PC medium-dendritic region. Intracellular recordings and perfusion of low Ca²⁺ medium identify these potentials as a fiber volley and EPSP characteristic of that described for parallel fiber input in cerebellar cortex and dorsal cochlear nucleus. Low frequency stimulation (5-20 Hz) evoked a pronounced frequency potentiation of the dendritic field negativity that recruited PC discharge. High frequency tetanization (90-200 Hz, 15 pulses; 7X) evoked a long-term potentiation of the dendritic negativity (83 +/- 23% above control) that persisted for a minimum of two hours. One slice demonstrated post-tetanic potentiation (19% above control) for 30 min while 3 slices showed no effect. Tetanic stimulation in low Ca²⁺ medium had no effect or evoked a depression of the parallel fiber volley for 20-30 min. The role of excitatory amino acids receptors in these forms of synaptic plasticity are under investigation.

454.3 DISTRIBUTION OF GLUTAMATE RECEPTORS IN THE ELECTROSENSORY SYSTEM OF GYMNOTIFORM FISH. L. Maler and D. Monoghan, University of Ottawa, University of California at Irvine.

Electroreceptor afferents of gymnotiform fish (Apteronotus leptorhynchus) project to the deep neuropil layer of a laminated hindbrain structure, the electrosensory lateral line lobe (ELL). The ELL projects to the nucleus praeminentialis dorsalis (Pd) which has feedback projections to the ELL, both directly to the ventral molecular layer of the ELL, and indirectly (via the caudal cerebellar lobe LC) to the dorsal molecular layer of the ELL. The circuitry of the ELL has been analyzed in detail and correlated with the spatial and temporal receptive field properties of the ELL pyramidal cells (PC). Bastian (1986) has demonstrated that the cerebellar feedback projection to the ELL can modulate the PC receptive fields.

We examined the distribution of glutamate receptor subtypes (AMPA, NMDA) in the ELL as well as in the midbrain. The AMPA receptors are found at low levels in the deep neuropil layer as well as throughout the molecular layer; AMPA receptors are also found at low levels in the superficial layers of the ELL, but are very dense in the dorsal cerebellar molecular layer. Kainate receptors are completely confined to the dorsal and cerebellar molecular layer, where they are very dense. NMDA receptors are present at high density in the molecular layer but only at low density in the deep neuropil, suggesting plasticity of the feedback loop.

454.4 MODULATIONS OF THE ELECTRIC-ORGAN PACEMAKER NUCLEUS OF GYMNOTIFORM ELECTRIC FISH BY PHARMACOLOGICALLY DISTINGUISHABLE PATHWAYS. M. Kawasaki, C.H. Keller, M. Helligenberg, UCSD, La Jolla CA 92039.

Both pulse and wave species of gymnotiform electric fish are capable of accelerating and decelerating the firing rate of their electric organ discharges (EODs) during social interactions. Modulatory inputs from the preganglionic neurons (PPN) to the pacemaker nucleus, which drives the electric organ with a regular rhythm, are responsible for these frequency modulations. Physiological recordings from the PPN, and application of pharmacological blockers to the pacemaker nucleus have revealed a difference in the mode of the modulatory blocks in wave and pulse species. In a wave species, Eigenmannia lineata, the increase and decrease of a tonic excitatory input to the pacemaker appears to be responsible for the acceleration and deceleration of the EOD frequency, respectively. In a pulse species, Hypomormynus brevirostris, on the other hand, direct GABAergic inhibition appears to decelerate the pacemaker, while glutaminergic excitation causes acceleration.
454.5

INTRACELLULAR LABELLING OF PHYSIOLOGICALLY DEFINED CELLS WITHIN A DICEPHALIC SENSORY-MOTOR INTERFACE OF WEAKLY ELECTRIC KNIFEFISH. C.D. Keller, M. Kawakami and W. Heiligenberg. UCSD, La Jolla, CA 92037.

The diencephalic complex of Eigenmannia's nucleus electrosensorius (nE) comprises a number of finely tuned neuronal populations for specific sensory-motor tasks. The nE can be subdivided into at least four distinct areas; three which receive electrosensory efferents of the torus semicircularis and one which contacts the electric organ discharge (EOD) frequency. nE controls of the EOD frequency, nE contains neurons responsive to jamming stimuli. A fourth neuron, receiving acousticolateral but not electrosensory input, may represent the acoustic component in the jamming avoidance response. Intracellular labelling of cells within each subdivision demonstrates different intranuclear patterns of connectivity and cell-specific efferent targets. We present data on a number of different cell types that are responsive to various electrostimuli. e.g., ongoing jamming stimuli, rapid modulations in jamming stimuli similar to courtship signals, and changes in orientation of the jamming stimuli. Some neurons responsive to jamming stimuli project to the vicinity of electric control areas of the diencephalon and likely contribute to the jamming avoidance response. Also, cell-types that may provide information about socially relevant stimuli project to hypothalamic targets and may thereby modulate endocrine or motivational systems of the CNS.

454.6

ULTRASTRUCTURAL STUDIES ON THE SYNAPTIC ORGANIZATION OF THE PREPACEMAKER NUCLEUS IN WEAKLY ELECTRIC KNIFEFISH. W. Heiligenberg, G.K.H. Zupanc, L. Maler and W. Heiligenberg. UCSD, La Jolla, CA 92037.

Large multipolar neurons of the diencephalic prepacemaker nucleus (PPn) of knifefish (Eigenmannia) have been shown to control a dibranchial behavior, namely abrupt frequency modulations ('chirps') of the otherwise constant electric organ discharges. By retrograde labelling of the PPn, we have now investigated the synaptic organization of the PPn.

We found two classes, each with two subtypes, of chemical synapses contacting PPn neurons: (1a) Symmetric type; (1b) Asymmetric type. These synapses are likely to mediate excitatory (class 1) or inhibitory (class 2) synaptic activity.

Electronoptonic junctions might be responsible for a joint depolarization of PPn neurons during chirping.

The chemical synapses are likely to mediate excitatory (class 1) as well as inhibitory input (class 2), each of which may be modulated by the release of the content of the dense-core vesicles (b-subtypes).

454.7

SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE PREPACEMAKER NUCLEUS OF ADULT KNIFEFISH CHANGES WITH SEXUAL MATURE.

G.K.H. Zupanc, L. Maler and W. Heiligenberg. UCSD, La Jolla, CA 92037; Univ. Ottawa, Ottawa, Ontario K1N 6N5.

Knifefish (Eigenmannia; Gymnotiformes; Teleostei) can modulate their otherwise constant electric organ discharge (EOD) frequency modulations ('chirps') of the otherwise constant EOD frequency. It has been shown that chirps are under the control of the prepacemaker nucleus (PPn). The morphology of PPn neurons undergoes sexual maturity-dependent changes which may be modulated by the release of the content of dense-core vesicles (b-subtypes).

In addition to these chemical synapses we also observed different types of electrical synapses, contacting PPn neurons: (1a) Asymmetric synapses contacting PPn neurons: (1b) Symmetric synapses contacting PPn neurons. They are likely to act in a hormone-like manner to provide information about socially relevant stimuli, e.g., in jamming avoidance behavior. The synaptic cleft is wider in class 1 than in class 2 synapses. In addition to these chemical synapses we also identified diencephalic-prepacemaker neurons, or it could act in a hormone-like manner to provide information about socially relevant stimuli.

454.8

SEX DIFFERENCES AND FREQUENCY MODULATIONS IN THE ELECTRIC ORGAN OF ADULT WEAKLY ELECTRIC KNIFEFISHES, HYPOPOMUS PINNICAUDATUS.

D.D. Hopkins, N.C. Comforţl and J. Bastian. Section of Neurobiology and Behavior, Mudd Hall, Cornell University, Ithaca, NY 14853; 1 Department of Neurobiology and Behavior, 2 Department of Zoology, University of Oklahoma, Norman, OK.

1) A newly described species, Hypopomus pinnicαudαtus from French Guiana has a sexually dimorphic caudal filament. Large sexually mature males have long "feathered" tail filaments, females have short cylindrical tails. The electric organ fills the caudal filaments in each.

2) The electric organ discharge (EOD) differs for the two sexes; male EODs average 1.6 ms in duration, female EODs are 1.1 ms. The power spectra of the male EOD peaks at 652 Hz while those from females peak at 915 Hz.

3) The peak-to-peak voltage of the EODs of 24 fish captured in the natural habitat using a monopolar electrode. The voltage declines according to the inverse square of the distance to the electrical null at the fish's mid-body. The voltage increases in proportion to the animal's body length. Females have a higher peak-to-peak voltage per unit body length than males. Males with damaged tails have a reduced voltage compared to unjured males of similar size. Males suffer more tail injury than females.

4) A computer model of the EOD suggests that the paradoxical reduction of the amplitude of the male's signal is a consequence of its increase in duration.

5) We hypothesize that sexual dimorphism in H. pinnicαudαtus has evolved under the influence of social selection for long duration pulses. The amplitude reduction resulting from the change in EOD duration is compensated for by the increase in electric organ length, number of electrocytes, and electrolyte size.

Supported by NIMH grant 37922 to CDH, NIMH M19573 to NC NIH NS19942 to JB.

454.9

SEX DIFFERENCES IN THE CAUDAL FILAMENT, ELECTRIC ORGAN, AND ELECTROCYTES OF HYPOPOMUS PINNICAUDATUS.

N. C. Comforţl, D.D. Hopkins, and A. Bao. Section of Neurobiology and Behavior, Mudd Hall, Cornell University, Ithaca, NY, 14853.

The South American electric fish Hypopomus pinnicαudαtus (sp. nov.) possesses an electric organ typical among gymnotiform pulse fish in its basic morphology: it is continually produced as a series of pulses, forming a quasi-sinusoidal waveform. The duration of each pulse is inversely related to EOD frequency; EOD pulse duration is longer in mature males than in mature females; and this difference can be mimicked by androgen treatment. The basis of the EOD is the simultaneous spiking of the electric organ electrocytes, thus electrocyte spike duration is expected to be highly correlated with EOD freq., and pulse duration, and to increase in response to androgen.

Recordings from intracellularly-stimulated electrocytes show two types of spikes with different dur., depending on stim. dur., both are similar in appearance to EOD pulses: in one type, the spikes are short (stim. <1.5 ms) and "short spikes" (stim. <1.5 ms) were measured from onset to end. Usually the onset of the short spike was obscured by stim. artifact, thus most were measured from peak to peak and multiplied by two. Some long spikes showed a pronounced hump in the falling phase not reflected in EOD pulse, which accounts in part for spikes being longer than EOD pulses. Long spikes ranged from 5.2 to 10.0 ms in pulse duration (4.9 to 8.1 ms) the short spikes were an average of 16.1% shorter than the long spikes (N = 13 fish). The relationship to EOD pulse dur. was r = 0.6 (long spikes), and r = 0.3 (short spikes). Spike duration: change with stim. dur. is also seen in vertebrate cardiac fibers, which often firing regularly-spaced spikes. In electrocytes, this may indicate that there is an ion conductance that is activated by a long dur. stim. Since only the long spikes are correlated with EOD pulse durude, the duration of long spikes is likely to be important in determining EOD pulse dur.

In addition, preliminary studies suggest that long spike dur. in electrocytes increases in response to adrenergic/noradrenergic treatment, resulting in the increase in EOD pulse dur. Similar results are seen in other electric fish species.


The Rattrayiformes include the Anguilliformes, the Ostariophysi, and the Gymnotiformes. The Gymnotiformes are characterized by the presence of a large mid-ventral sinus or sinus venosus, which is connected to the heart by a large ventral aorta and a pair of lateral aortas. The midventral sinus is a common feature of the Gymnotiformes, and it is believed to play a role in the regulation of blood pressure and the distribution of blood to the body tissues. The Gymnotiformes also have a well-developed brain, which is thought to be responsible for the complex behaviors observed in these fish. The Gymnotiformes are also known for their unique electrical organs, which are used for communication, navigation, and predation. The electrical organs are composed of a large number of modified muscle cells, which can generate a strong electric field. These electric organs are thought to be controlled by a complex nervous system, which is responsible for the generation and modulation of the electric signals. The Gymnotiformes are also known for their unique reproductive behaviors, which are thought to be facilitated by the presence of a large midventral sinus and a well-developed brain.
454.17


Single spikes and multi unit activity in response to weak (<75 pV) electric field stimuli delivered via a 3 cm dipole perpendicular to the skin surface in the receptive field were recorded with glass micropipettes from the granule cell layer of the cerebellum of a disembowled thornback guitarfish (Diplodus holocanthus). The single unit spikes apparently come from large cells, presumably interneurons.

Differences in temporal response properties between two discrete electroreceptive regions in the caudal lobe of the cerebellum correspond to distinctively different aspects of electric field stimuli. The electroreceptive afferent innervation of these areas is unknown.

Supported in part by NIH NS06814 to JGN.

454.18

ELECTRORECEPTIVE AND PROPRIORECEPTIVE REPRESENTATIONS IN THE DORSAL GRANULAR RIDGE OF SKATES. R.A. Conley* and D. Baudisch. Wesleyan Univ, Middletown, CT.

Physiological responses and receptive fields of proprioceptive and electroreceptive neurons in the dorsal granular ridge (DGR) of Rajiformes have been studied in decerebrated, cutaneous fish. DGR is part of the vestibulolateral center, a large diffuse network of neurons topographically parallel fibers to the electrosensory dorsal octavolateralis nucleus (DON) in the medulla. DGR probably has a major influence on primary electroreceptive inputs to the brainstem, and projects to the associated central nervous system. The DGR was studied in 178 neurons recorded in a blind mapping study, 99 (56%) had their discharges modulated with whole body and 79 (44%) were unaffected by fish displacement, but responded to EC electric fields. No bimodal units were encountered. Units were characterized as proprioceptive since they had a distribution in the area of the body oriented in proportion to fin displacement, although some units had a phasic component during fin movement. For each proprioceptive unit there was a limited area of sensitivity along the fin margin. The electroreceptive units had large ipsilateral receptive fields, whose exact boundaries were often unclear. But at threshold, nearly all units had a best area within the receptive field. In general, both proprioceptive and electroreceptive units showed a progression of receptive fields from posterior to anterior body along the caudorostral axis of DGR. DGR forms a topographical projection onto DON such that caudal DGR projects to dorsal DON and rostral DGR projects to ventral DON. As a caudal to rostral body map is present along the dorsorostral axis of the DON, these results suggest that DGR’s projection onto the DON is homotopic.

454.19

AUTORADIOGRAPHIC LOCALIZATION OF DIHYDROTESTOSTERONE CONCENTRATING NEURONS IN THE BRAIN OF THE OYSTER TOADFISH. M.L. Fine and D.A. Keefer*. Deps. of Biology, Virginia Commonwealth University, Richmond, VA 23284 and Loyola College, Baltimore, MD 21210.

Steroid-concentrating neurons in the brainstem have been reported in two teleosts, the toadfish and a moray, both of which produce courtship sounds. In this study "3H DHT-labelled neurons were found in the forebrain and brainstem. Positive sites included the supramammillar nucleus of the ventral telencephalon and the base of the lateral portion of the dorsal telencephalon. Small numbers of cells were labelled sporadically in the parvo- and magnocellular pretectal nucleus and in the ventral and dorsal hypothalamic nucleus. The caudal hypothalamus had a densely-labelled triangular-shaped nucleus and nearby scattered cells. The periventricular portion of the posterior tuberculum of the thalamus was also labelled. Scattered heavily labelled cells were present throughout the basal optic tectum and superficial torus semicircularis. In the medulla a dorsal accumulation of cells was present beneath the cerebellum on either side of the fourth ventricle rostrally and in reticular neurons caudally.

Compared with an earlier study with "3 estradiol, estrogen labelling was generally more prominent in the forebrain, and with the exception of the torus semicircularis and tectum, no estrogen target neurons have been found in the brainstem. Supported by NIH M083921 and NSF PCM830914.

454.20


The electroreceptive lateral line lobe (ELL), the first-order electroreceptive processing station, receives major descending inputs in addition to afferents from the electrosensors. The nucleus praeeminentialis (NP), which receives electroreceptive inputs from the ELL and the torus semicircularis, is the principle source of descending input to the ELL. NP neurons were studied using extracellular, single-unit and intracellular recording methods and Lucifer yellow labelling for anatomical identification focusing on two categories of neurons.

Two categories of NP neurons display relatively different kinds of activity. First, nonfastigial NP neurons respond to stepwise changes in electric organ discharge (EOD) amplitude phasically and are insensitive to sinusoidal modulations of EOD amplitude of frequencies exceeding 30 Hz. Previously reported as the most common NP cell type, these neurons respond vigorously to electrolocation targets moving along the contralateral side. Ipsilateral targets cause little or no response, hence the ST neurons receive excitatory input from the contralateral ELL. We found two populations of ST neurons, one responds to contralateral increases in EOD amplitude while the other responds to decreases, there were no differences in morphology or projection pattern.

Tuffted neurons influence the ELL indirectly via their projection to the posterior eminentia granularis (EGP). These neurons spatially summate EOD activity over the contralateral ELL, thus creating a complex waveform to air-mediated sound. This auditory EPSP has been recorded from other reticulospinal neurons. [Supported by NIH Grant NS22621 and OCAST #1669].

454.21


The orientation of juvenile rainbow trout (Salmo gairdneri) (age approx. 6 months) was tested under: (1) normal, (2) null, and (3) rotated magnetic field conditions. In the normal geomagnetic conditions, the horizontal component of the magnetic field vector is involved in such orientation, and (3) normal magnetic orientation is not possible without the dipoles which constitute the lateral zone of the dorsal telencephalon, a piscine hippocampal homolog.

We find that the activity of the neuropil modulates the spontaneously oscillating field potential of the procreational (PC) lobe. Using a nose-brain preparation, brief pulses of moist air or 2-ethyl-3-methoxypyrazine were delivered to the nose while the PC oscillation was recorded with a saline filled electrode in the PC neuropil. Odor pulses modulate the spontaneous oscillation in a transient DC and changes in frequency and waveform of the oscillation. Air pulses also modulate the oscillation, but to a lesser extent than odor pulses. Simultaneous histofluorescent recordings from distal cell body and basal neuropil regions show in phase oscillations of opposite conduction. A suction electrode placed over the intact lobe provides a convenient measurement of whole-loba oscillation.(Fig. 1a). Intracellular recordings show that individual neurones generate action potentials during the depolarizing phase of large membrane potential oscillations (Fig. 1b). It will be of great interest to determine if there exist odor-generated spatial patterns of excitation in PC neurones.


In the isolated CNS of the pond snail, Lymnaea stagnalis, the feeding rhythm persists at a slow rate, through the interaction of the N1, N2 and N3 premotoneurones (Elliott, C.J.H. & Benjamin, P.R., J. Neurophysiol. 35:1393, 1972). Phenylmethylamine (PMTA, 0.5mM in normal saline) blocks all synaptic output from the N1 neurone and rhythmic activity then appears to be confined to these interneurones. Hexamethonium or atropine (0.5mM) block the EPSPS (but not the IPSPS) produced by the N1 interneurones. These antagonists also abolish the spontaneous rhythm and slow the rhythm elicited by the stimulation of a modulatory interneurone, the S0. These results confirm that the N1 neurones are cholinergic (Elliott, C.J.H et al., Symp. Biol. Hung. 36:597, 1988) and suggest that recurrent inhibition between the N1 and N2 neurones plays an important part in rhythm generation.


Molluscan buccal ganglia have been extensively used to study the neural basis of feeding behavior. However, detailed information about the underlying neural circuits is lacking for most families of molluscs because of their size and complexity. We have been investigating the buccal ganglia in the nudibranch Melibe levona, because their simplicity (30 neurones) makes them amenable to analysis at the cellular level. The goals of our research are to: 1) describe the feeding behavior in detail; 2) make preparatory and functional recordings from distal cell body and basal neuropil regions show in phase oscillations of opposite conduction. A suction electrode placed over the intact lobe provides a convenient measurement of whole-loba oscillation.(Fig. 1a). Intracellular recordings show that individual neurones generate action potentials during the depolarizing phase of large membrane potential oscillations (Fig. 1b). It will be of great interest to determine if there exist odor-generated spatial patterns of excitation in PC neurones.


The food-induced arousal state in Aplysia has multiple manifestations, including changes in consummatory behaviors (increased rate and magnitude of biting), appetitive behaviors (head-up posture), autonomic responses (increased heart rate), and defensive responses (depressed head withdrawal). To determine if these responses might be controlled by a central arousal system, we examined the neural control of appetitive arousal, which appears to precede all other aspects of food-induced arousal. The central pattern generator of the pedal ganglion which project to the pedal ganglion was studied. A unique cerebral- pedal interneuron, CPA, was identified, which activates numerous neurones in the pedal ganglion. The CPA has either poly- or monosynaptic connections to more than 50% of the pedal ganglion neurones, including a large population of neck motor neurones. Fixing the CPA evokes bilateral contractions of neck muscles, which could serve to lift the head into the animal feeding posture. The activity of the CPA evokes the pedal ganglion provides excitatory drive to a number of other neurones which mediate various aspects of the food arousal state, including the MCCs, which modulate biting, the CBSIs, which drive the biting program, and the RB cells, which increase heart rate. Finally, pedal ganglion activity driven by the CPA inhibits cerebral Bn cells. Removal of the CPA neurones from the circuitry by hyperpolarizing them bilaterally strongly reduced the effects of food on both the MCC and the Bn cells. Therefore, it appears as if activity of the CPA may be necessary for some, and perhaps most aspects of the responses associated with the food-induced arousal state. It is possible that the CPA functions in the role of a command neuron or command element that evokes a behavioral state in the animal, rather than a specific behavior.


In two studies Aplysia c. were fed from 50 to 110+ days post-hatching on either a protein & tryptophan rich Gracil·aria or a low protein & tryptophan Enteromorpha diet. At 10+ days a·plysia were tested for short-term sensitization, using escape locomotion following tail shock. The experiment showed that odor stimulation of the oral hood initiated by the current required for tail withdrawal, followed by the sensitizing shock and two more weak shocks. Number of steps in the tail shock zone, following each successive shock, is not increased during subsequent analysis. Then animals were dissected and their gut contents analyzed. All animals captured food normally and accumulated comparable numbers of prey, indicating that the buccal ganglia do not play a role in food capture. Analysis of the distribution of food in the gut revealed that in lesioned animals up to 80% of the food ingested remained in the anterior esophagus. In contrast, in both control groups the majority of the food was in the stomach. These data indicate that the buccal ganglia in Melibe control swallowing, but not the capture of food. (Supported by grants from CURF to A. V, UROP to J.T., and Hubbard W.W.)

545.6 SEROTONIN AFFECTS BEHAVIORAL STATE AND FEEDING BEHAVIOR IN THE SNAIL, HELIX ASPERNA. BUT HAS NO EFFECT ON SEXUAL BEHAVIOR. S.A. Adamo and R. Chase. Department of Biology, McGill University, Montreal, Canada H3A 1B1.

The degree of activity in H. asperna can be used as a measure of the snail’s behavioral state. Behavioral state can be taken to represent an underlying level of general arousal, as has been done for Aplysia and Pleurobranchaea. Injections of serotonin (10 -7 moles/kg body weight) increased territorial and copulatory behaviors in H. asperna (p < 0.01), which is consistent with the effects of serotonin in Aplysia and Pleurobranchaea (Palovcik et al., Behav. Neural Biol., 35:383, 1982). Handling the animals had a similar, but smaller effect (p < 0.05). Serotonin injections also increased feeding behavior as compared to controls (p < 0.05). Serotonin had no effect on sexual arousal (monitored by observing changes in the snail’s genital eversion). Serotonin also had no effect on sexual prorivity (i.e. the frequency of mating, p >> 0.05). These data suggest that, in H. asperna, mechanisms that mediate sexual behavior are distinct from those that control feeding and behavioral state. Therefore, sexual behavior is one ‘motivated’ behavior that does not appear to be significant post-gastric central serotonergic system (Palovcik et. al., 1982).
Mechanoreceptive Inputs Produce a New Type of Nonassociative Learning in Tritonia, D. Cawthorpe & K. Lukowiak (SPONSOR: K. E. Cooper). University of Calgary, Calgary, Alberta T2N 4N1 Canada. TRITONIA C.A.LIFORNICA is a model system used in the study of the neural and biochemical basis of behavior. Both the central nervous system (CNS) and peripheral nervous system (PNS) have been shown to act in an integrated manner to mediate gill withdrawal behavior (GWB). This GWB has been shown to undergo both associative and non-associative forms of learning. The most recent studies have concentrated upon the role of the CNS in learning due to the easier accessibility of the CNS pathways. As yet little is known about the PNS. The particular sites of action and the identity of neurotransmitters and/or neuromodulators in the PNS remain largely unknown. In response to this gap in our knowledge about the PNS, a primary culture of dissociated gill muscle fibers has been developed. This provides an opportunity to survey the neurotransmitters and neuromodulators known to act in the PNS. We report here that the endogenous peptide, FMRFamide, acts directly on isolated gill muscle fibers to bring about contractions at concentrations (10 nM) which have been shown to increase the GWR amplitude and prevent habituation in the in vitro reduced gill preparation (Cawthorpe et al., 1988; Higgins et al., 1989).

455.9 NEUROSECRETORY CELL R15 IN APLYSIA ACTIVATES RESPIRATORY PUMPING, MOTONEURON L7, AND THE HERMAPHRODITIC DUCT. A. Alevizos, K. R. Weiss, B. C. Weiss & K. Koester, Center for Neurobiology & Behavior, Dept. of Psychiatry, and Dept. of Cellular and Biophysical, Columbia University, N.Y., N.Y. R15 is a neurosecretory cell in the abdominal ganglion, thought to be involved in water and salt secretion. We have shown that central and peripheral synaptic effects on the abdominal ganglion - elicits unidentified cells of the left lower quadrant and the L7 vive vacuolar cells. We have shown that the modulation of effects of R15 and its bioactive (1-6 peptide) on respiratory pumping) 2) motoneuron L7 and 3) peristaltic activity of the large hermaphroditic duct. Respiratory pumping consists of transient, synchronous pumping actions of the mantle organs and pericardial. It is driven by a network of about 30 electrically and chemically coupled interneurons in the abdominal ganglion. We found that R15 is hyperpolarized for prolonged periods of time (1.5 - 2 hours) to prevent it from firing, and then allowed to burst spontaneously for 5-60 minute intervals, which gradually decays over 20 to 60 minutes. In the same long hyperpolarization protocol, R15 also excited the multimodal cardio-respiratory desensitization that lasts for hours, and all the effects of R15 can be cross-desensitized that lasts for hours, and all the effects of R15 can be cross-


We constructed ensembles of Aplysia LUQ neurons in culture. These cells form chemical connections and may couple electrotonically. Their electrical activity was measured with multispot optical recording techniques and voltage-clamp recordings (Fiscus et al., 1987, J. July 1989). These techniques allow us to record for prolonged periods with a good signal-to-noise ratio and negligible photobleaching (2 hr, S/N ≥ 10, respectively). The extended outgrowth of the neurites caused the axon to move the activity of many cells. We show how individual spike trains were reconstructed from the spatial distribution of timing and amplitude information in the ensemble records. The effective connectivity was determined from the spike trains (Figure). Weak interactions could be deduced from repeated measurements. (Supported by AT&T and NS 19824)

455.12 SYNAPTIC INTERACTIONS IN ASSEMBLIES OF CULTURED APLYSIA NEURONS. II. Dynamic patterns of effective connectivity. T. D. Parsons, B. M. Salzberg, A. L. Obaid, F. Racusin-Delehanty, and D. Klinefeld, AT&T Bell Laboratories and University of Pennsylvania School of Medicine.

We studied the effective connectivity between LUQ neurons co-cultured as 4 to 6 cell ensembles. These cells form biphasic chemical connections, with inhibition followed by excitation, as well as monophasic inhibitory connections. The effective connectivity was determined (preceding abstract) when the neurons were quiescent (10 nM) and when they were firing at ~ 5 Hz (10 nM). Under conditions of quiescence the neurons exhibited excitatory interactions (Figure A). In contrast, the same ensemble excited inhibited dendrites (Figure B). The change in effective connectivity could be understood in terms of the underlying monophasic connections. The excitatory interactions were much weaker than the inhibitory synaptic interactions (Figure B).

FMRFamide ACTS DIRECTLY ON ISOLATED APLYSIA GILL MUSCLE CELLS. D. Cawthorpe & K. Lukowiak (SPONSOR: R.K. Cooper). University of Calgary, Calgary, Alberta T2N 4N1 Canada. APLYSIA CALIFORNICA is a model system used in the study of the neural and biochemical basis of behavior. Both the central nervous system (CNS) and peripheral nervous system (PNS) have been shown to act in an integrated manner to mediate gill withdrawal behavior (GWB). This GWB has been shown to undergo both associative and non-associative forms of learning. The most recent studies have concentrated upon the role of the CNS in learning due to the easier accessibility of the CNS pathways. As yet little is known about the PNS. The particular sites of action and the identity of neurotransmitters and/or neuromodulators in the PNS remain largely unknown. In response to this gap in our knowledge about the PNS, a primary culture of dissociated gill muscle fibers has been developed. This provides an opportunity to survey the neurotransmitters and neuromodulators known to act in the PNS. We report here that the endogenous peptide, FMRFamide, acts directly on isolated gill muscle fibers to bring about contractions at concentrations (10 nM) which have been shown to increase the GWR amplitude and prevent habituation in the in vitro reduced gill preparation (Cawthorpe et al., 1988; Higgins et al., 1989).
EVIDENCE FOR ASSOCIATIVE LEARNING IN THE NEMATODE C. ELEGANS N. Kumar*, M. Williams*, J. Culotti, and D. van der Koop. (SPON: A. Roach), Dept. of Anatomy and 1Medical Genetics, Univ. of Toronto, Toronto, Canada, MSS 1A8.

A simple in vivo model for associative learning and memory offers many advantages in that it is not as difficult as invertebrates. We have developed a classical conditioning paradigm using chemosensory responses to the conditioned stimuli (CS) Naa(CH++) and Cu (Ni(+) ions, and E. coli (a food source) as the unconditioned stimuli (US) for C. elegans. CS levels were balanced such that naive animals displayed equal preferences for either CS. The animals were then trained by exposing them to one ion paired with the UCS (CS+) followed by exposure to the other ion (CS-) for 30 s. The experiment was conducted with or without the presence of the UCS (CS). The two experiments were performed separately.

After 48 h of training, the level of learning was no longer significantly different (56.5% vs 39.5%) for controls. The results indicate that C. elegans is capable of associative learning and should be useful in a mutational analysis of the molecular basis of learning and memory.


Operant conditioning is the least well investigated learning procedure in animals with simple nervous systems. In particular, lever press and other traditional operant behaviors studied in vertebrates have been difficult to obtain in invertebrate species. We have developed an operant chamber for the green crab (Carcinus maenas) in which pressing a lever produces food reward. The operant chamber, submerged in seawater, contained a plastic rod attached to a microswitch and food was dispensed from a tube positioned at mouth level. In one series of experiments, one bar was present on either side of the food tube. Pressing one lever (S+) was reinforced, whereas the other (S-) was not. Experimental animals pressed S+ with an average rate of 6.6/min over the first 5 min, with rates slowly slowed down. Response rate was averaged 3/min and rapidly dropped out. Typical response times were 7 s.

The contingency of the lever with the food dispenser turned off after 10 s and the bar was then present on the other side of the food tube. By 27 hours post-training the level of learning was no longer significant (40.8 ± 2.1%). Thus, the C. elegans is capable of associative learning and should be useful in a mutational analysis of the molecular basis of learning and memory.

EFFECTS OF OCTOPAMINE ON UNITARY EPSPS IN A FIRST ORDER INTERNEURON OF CRAYFISH LATERAL GIANT ESCAPE CIRCUIT. J. Bustamante* and F. Keene. Dept. Physiology, Faculty of Medicine, Universidad Complutense de Madrid and Dept. Psychology and Brain Research Institute, UCLA.

The excitability of the crayfish giant lateral giant escape reflex can be increased for hours by traumatic stimulation, LTP-like regimens, and exposure to octopamine. Using the octopamine augmentation as a possible model of the other types, we examined octopamine's effect on the EPSPs in L1. Octopamine exposure caused EPSP amplitude to increase (150%, SD 60%) within minutes, but recovery required hours of washout. Augmented EPSPs had more variable amplitudes (control CV of 7.8%, SD 6.4%, 80% 18%) and decayed more slowly (~4 slows half full 12 s) significantly by 5% than controls. The results suggest that the octopamine brings into increased action a population of previously unexpressed synaptic transmission sites that have relatively variable release and slow decay kinetics (t approx 20 compared to 6 for control).

Supported by NSC Fellowship, Spain (JB) & NIH grant NS 08108 (FK).


To understand how genes specify an innate behavior, we are genetically dissecting male-mating in C. elegans. Since the hermaphrodites are self-fertilizing, mutations that affect or even eliminate male-mating behavior can be isolated and maintained for study. By mutagenizing with EMS a strain that segregates 40% XO males via non-disjunction of the X-chromosome, we have isolated mutants that have no immediately visible anatomical defects yet are propagation defective (Cod). We predict that such mutants might be blocked in mating behavior at any one of a number of necessary steps: attraction to hermaphrodites, response to contact with hermaphrodites, location of the vulva, insertion of spicules into vulva, and sperm transfer.

Of the mutants isolated thus far, the majority (6 of 9) appear to be unable to insert their spicules, indicating a muscle or motor defect. One responds poorly to contact with hermaphrodites and two are unable to locate the vulva. These failures may indicate underlying sensory defects. In addition, two mutant strains give males that sire very few progeny, indicating either a defect in sperm transfer or sperm potency.

POSTSYNAPTIC INHIBITION OF THE LATERAL GIANT NEURON DURING RERAINT-INDUCED SUPPRESSION OF CRAYFISH ESCAPE. E.I. Wu and F.B. Krasne. Neuroscience Program, Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

The suppression of the crayfish lateral giant (LG) escape response by restraint is at least partially mediated by decreased synaptic transmission onto the LG. We have previously reported a 15% decrease in root-evoked LG postsynaptic potentials (PSP). We now report comparable decreases in both the electrical unitary PSP from the identified sensory interneuron, interneuron A, and in polarizing pulses injected into the electrically coupled contralateral LG. In contrast, axonally applied LG polarization is only decreased about 14%, thus suggesting that the LG receives a conductance increasing postsynaptic inhibition during restraint that operates distally in the dendrites. This site of action is different from that of recurrent inhibition of this cell, and is similar to that of another recently reported form of postsynaptic inhibition of the LG, post excitatory inhibition. Supported by USPHS grant NS08108 (FK) and a Predoct. NSF Fellowship (EV).

ALTERED SYNAPTIC TRANSMISSION AND MEMBRANE CURRENTS IN THE DROSOPHILA LEARNING MUTANT dunce. Yi Zheng & Chun-Fang Wu (SPON: S. Singh). Dept. of Biology, University of Iowa, Iowa City, IA 52242.

Several proposed models for cellular mechanisms underlying learning and memory involve regulation of neuronal plasticity by second messenger systems. A general question is whether the conserved set of genes controls the molecular regulatory mechanisms exclusively required for learning and memory. Mutations of the dunce (dnc) locus in Drosophila, which cause reduced phosphorylated CREB activity in elevated cAMP levels, reveal deficiencies in learning and memory paradigms but not in other behavioral tests. It is not clear whether dnc mutants affect synaptic plasticity and membrane currents and whether the effects are confined to subsets of neurons or more generally excitatory cells.

With the two-microelectrode voltage-clamp method, we examined neurouromuscular transmission and membrane currents in larval body wall muscles preparations. The excitatory junctional currents (ejes) in normal larvae, but not in dnc. At 0.4 Hz stimulation for 5 min, potentialization, which lasts for minutes, was observed in normal, but not in dnc. Furthermore, we found an increase in the amplitude, both transient and Ca++ current in dnc muscle membrane. Interestingly, modification of synaptic efficacy and of these two currents has been implicated in other invertebrate model systems for learning and memory. Supported by NIH grants NS 18500 and NS 26528.
SEROTONIN DEPLETION REDUCES SEVERAL FORMS OF BEHAVIORAL FACILITATION IN THE LEECH. T. Karrer, J. Erlich*, N. Boulis and C. Sahley*, Dept of Biology and Psychology, Yale University, New Haven CT. 06511

We have previously shown that the shortening reflex of Hirudo medicinalis undergoes two forms of facilitation, dishabilitation and sensitization (Boulis and Sahley, 1988). Other labs have suggested a role for serotonin (5HT) in facilitation in Hirudo (Berton et al., 1987; Catarsi et al., 1987; Lockery et al., 1987). For this reason, we tested the role of 5HT in facilitation of the shortening reflex. Specifically, we depleted 5HT and observed the subsequent effects on dishabilitation, sensitization and facilitation. SHT depletion and histological corroboration were done using a modified version of Lent's technique (1982). Two weeks after the injections half of the leeches were habituated to a low level shock stimulus (2.5-3.5V) applied to the skin of the leech, followed by dishabilitation trials using a higher level shock (10V) also to the skin. The remainder of the leeches received sensitization trials using a 10 V stimulus prior to undergoing the habituation regimen. Glyoxylic acid staining revealed that 5HT was always depleted in the Retzius cells of experimentals. Depletion of the smaller 5HT neurons was variable.

Behaviorally, 5HT depletion attenuated dishabilitation and prevented sensitization. In addition, the initial facilitation shown by controls early in habituation training was absent in experimentals. These results suggest that 5HT, and perhaps Retzius cells play an important role in facilitation of the shortening reflex of Hirudo.

A DETERMINISTIC MODEL OF ION CHANNELS WITH ACTIVE DENDRITES

G. T. Bartus* and R. F. Thompson (SPON: W.L.Brycey) .

Neural, Informational, and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089.

The objectives of detailed modeling of Purkinje cells include reproduction of known physiology to gain insight into underlying mechanisms, exploration of input/output relations to put constraints on possible functions, and integration into a circuit level model.

The simulation code based on the compartmental method allows for the specification of arbitrary cell morphology, distribution of ion channels, and stimuli. The following active ionic conductances have been implemented using Hodgkin-Huxley type equations: 1) the ubiquitous voltage sensitive inactivating Na+, 2) a slower spike-generating Ca++, 3) a voltage sensitive K+, 4) a slow voltage and Ca++ dependent K+, and 5) a non-inactivating Na+ conductance.

Physiological data was better reproduced in Purkinje cell simulations with Na+ currents located in the soma and Ca++ related currents in the dendrite. Several conductance variations showed bursting behavior. Tests of synaptic input/cell firing output gave a near linear relation when somatic conductances dominated dendritic conductances.

Supported by NSF (BNS-8718300), the McKnight Foundation, and ONR (N00014-88-K-0112) grants to RFT.

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HH CHANNELS ALTER SUMMATION OF SYNAPTIC INPUTS IN A MODEL NEURON

J.W. Moore, M.L. Hinds*, & J.K. Gobbel*


We have examined the effect of insertion of Hodgkin-Huxley (HH) channels in passive neuron models of Rail. Computer simulations were carried out on a PC using a new simulation package CABLE (Int. J. Biomed. Comp., in press) We have followed, with minor changes, Dodge's (1979, The Neurosciences, 4th Pgm., 439) assignments of morphology and HH channel densities for a motor neuron.

In testing for spike generation by "temporal summation" of two synaptic inputs, we found their relative timing to be more important than their amplitudes when Na & K channels were present. Contrary to the conventional notion (based on passive membranes and often used in nerve networks) that a spike can be produced by summation of EPSP's to a level exceeding a constant threshold, we find such synaptic inputs will sum to generate a spike only during a relatively brief period when both are rising.

For an "excitatory" synaptic input, the presence of Na channels causes a greater depolarization than in a purely passive neuron. The presence of K channels (with a slowly increasing and decaying conductance) both speeds the return toward rest and reduces the effectiveness of a following EPSP. This inhibitory effect is strong enough to prevent the generation of a spike, even by a train of closely spaced EPSP's.

Conversely, because IPSP's decrease gK and thus make the membrane potential more sensitive to current, they can have an excitatory effect for a long period following their peaks.

Neurons with voltage-sensitive channels offer much richer repertoires of integration than do passive membranes.

COMPUTATIONAL MODELS OF OLFATORY RECEPTOR NEURONS: COMPARISON OF CONVENTIONAL AND WHOLE-CELL DATA.

E. Pongracz*, S. Firestein and G.M. Shepherd, Section of Neuroanatomy, Yale University Medical School, New Haven, CT. 06510.


We have analysed these discrepancies by constructing computational models of the receptor neurons in the two recording situations. The simulations have run on SABER (Analogy, Inc.), a general simulator program that permits rapid exploration of linear and non-linear parameters.

Analysis of the models has shown that a leak around the electrode, coupled with differences in cell morphology, can account for the lower values of Em, Rm and A in the conventional recording mode. Peaked exponentials indicate an electrotonic length of the dendrite-cilia of less than 0. The results support studies in motoneurons demonstrating the importance of electrode leak in assessing membrane properties.

We are extending the models by incorporating transduction-gated conductances in the cilia that are activated by sensory stimulation, and voltage-gated conductances in the soma and axon that generate the impulse response.

There has been indirect evidence that differential conduction, which is based on spike frequency, occurs at branch points of particular axons in crayfish and also in leech. However, the crayfish motor system and leech sensory systems appear to reflect different mechanisms. In both systems differential conduction occurs after an increase in threshold following a period of prolonged activity. However, in crayfish the prolonged activity is accompanied by a slight depolarization which in leech prolonged activity is accompanied by hyperpolarization. In addition, antihyperpolarization relieves conduction failure in crayfish, but mimics conduction failure in leech. Furthermore, inactivating the electrogenic pump enhances conduction failure in the crayfish preparation, but relieves conduction failure in the leech preparation.

A computer simulation has been used to explore the hypothesis that both differential conduction and the differences in excitability changes in the two preparations can be explained by different axonal-specific densities of electrogenic pump sites. The mechanism to account for differential conduction involves sensitivity of the electrogenic pump to external potassium and internal sodium concentrations, which causes the pump to activate sooner in a thin branch than in a thicker branch. Consequently, there can be differences in excitability in two branches of the same axon, even though the density of pump sites per unit area of membrane is uniform. The differences in equilibrium potential accompanying prolonged activity in the two preparations can be accounted for by different overall densities of pump sites in the leech and the crayfish preparations with the density in the leech preparation being much higher.

Supported by NIH grant 00126, NSIMS T32-GM07494, & Interactions Co., Cambridge, MA.

ACTION POTENTIAL DURATION IN A RAT PITUITARY CELL. A. Alonso.

IONIC CURRENTS IN RAT LACTOTROPHS. D. Janigro, G. Maccaferri, and J. H. Heidbreder. Dept. of Pharmacology, H San Raffaele, Milano, Italy 20132 Milan, Italy.

Ionic currents were investigated in purified rat lactotrophs in primary culture. Patch-clamp experiments using the whole cell configuration revealed large potassium currents elicited from a holding potential of -110 mV when recordings were performed in standard extracellular solution (in mM: 150 NaCl, 5 KCl, 2 CaCl2, 2 MgCl2, 5 HEPES, 5 Glucose). An outward current required calcium influx for its activation since removal of extracellular calcium ions abolished this component thus permitting the study of a long lasting current. After blockade of potassium channels, with intracellular CsCl (130 mM), the outward current was very small and high threshold calcium currents were elicited from a holding potential of -100 mV and +40 mV, respectively. Partial inhibition of the inward currents was obtained after cell exposure to amiloride (250 µM) and TTX (120 µM).

These results confirm the existence of both calcium and potassium conductances in rat lactotrophs.
A MODEL OF EXOCYTOSIS BASED ON THE DEPLETION OF IONS FROM THE SPACE BETWEEN VESICLE AND PLASMA MEMBRANE.

We propose the following revised version of a model for the mechanism of calcium-dependent exocytosis:

1. Increased cytosolic calcium concentration causes opening of a calcium-activated potassium channel located in the secretory vesicle opposite the plasma membrane. Opening of the channel causes influx of potassium ions into the vesicle. This tends to deplete salt concentration in the space between the vesicle membrane and the plasma membrane.

2. In presence of calcium, this space is extremely narrow, limiting replacement of lost ions. Because of net ion loss, the space becomes hypertonic and water leaves, causing the two membranes to fuse.

Because of the small size of the space which becomes hypertonic, only a very small number of molecules leave. Therefore, it is not possible for sodium molecules to take the place of potassium in lowering the osmolality of the space. Thus, the experimental evidence that replacement of cytosolic potassium by sodium or by sugar has no effect on the rate of exocytosis, does not necessarily contradict the model.

456.16

ANTI-CALMODULIN AGENTS (ACA) AFFECT DEPOLARIZATION- AND CALCIUM IONOPHORE-INDUCED VASOPRESSIN (AVP) RELEASE BY AN ALTERNATIVE MECHANISM. N. F. Rossi. Dept. of Medicine, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Calmodulin has been implicated in transducing the effects of calcium on synaptic transmission and hormone release, including osmotically-stimulated AVP release. ACA block AVP release secondary to inhibition of calcium-calmodulin interactions, these drugs should inhibit AVP release to stimuli increasing calcium influx via different mechanisms. Hypothalamo-neurohypophysial complexes (HNC) in culture were exposed to 7μM ionomycin, 1μM Bay K 8644, or 7μM veratridine (Bay K 8644) and 0.01 M CaCl₂. These results are consistent with the hypothesis that ACA inhibit AVP release by membrane stabilizing effects rather than by antagonizing calcium-calmodulin in HNC. Depolarization initiated by sodium influx may stimulate sodium-calcium exchange independent of slow calcium channels.
456.17


The brain of the turtle Chrysemys picta exhibits remarkable anoxia tolerance. One explanation for this phenomenon might be that turtles have low metabolic rates both during normoxia and anoxia, allowing glycolytic ATP production to satisfy neuronal energy requirements. Normoxic metabolic rate was measured using the [3] 1-deoxyglucose method in vivo. The turtle brain consumed 0.055 mg/kg/min at 23°C. 1/12 the published glucose uptake rates for rat brain. Rat brain homogenates exhibited a 2-fold greater Na+,K+-ATPase activity on average as compared to the turtle brain at the same temperature. These data suggest that ion leakage and pumping in the normoxic turtle brain is less than that in the rat brain. To obtain a measure of ion leakage, intracellular recordings of cortical pyramidal neurons in vitro were performed during normoxia and anoxia. Time constants, which reflect the product of membrane resistance and capacitance, did not increase with anoxia, but were significantly greater in turtle than rat, suggesting lower normoxic ion fluxes in the turtle brain. In contrast to the results with time constants, apparent input resistance decreased with anoxia. Current efforts are directed towards determining whether increased synaptic activity is responsible for this change.

456.18


The effects of anoxia on the CNS have been extensively studied in gray matter, but much less is known about the consequences of oxygen deprivation in white matter. We have used the isolated rat optic nerve (RON), a typical central white matter tract, to study the pathophysiology of anoxic injury in mammalian white matter (Davis and Ramsay, Soc. Neurosci. Abst., 13:364, 1987). The area under the compound action potential, recorded with suction electrodes, was used as an indicator of functional integrity of the RON, allowing quantitative evaluation of recovery after anoxic insults and of the effects of various interventions. RONs from adult (50-70 days) animals were dissected free, placed in a perfusion chamber and superfused at 60 mm Hg oxygen for a 60-minute period of anoxia in a N2/CO2 atmosphere. We confirmed previous findings of 20-30% recovery after anoxia (as judged by the area under the compound action potential) when the preparation was perfused with standard solution containing 2mM Ca2+; reduction of external Ca2+ to 0 mM greatly improved recovery (>80%). On the assumption that Ca2+ flux across the membrane is a critical factor responsible for irreversible anoxic injury, an assay was made to measure with its presumed entry through conventional voltage-sensitive L-type Ca2+ channels. Pre-treatment with nimodipine (1-10µM), an organic Ca2+ channel blocker, did not significantly improve recovery. Likewise, divalent cations such as Co2+ (1.4 mM) and Zn2+ (0.1-1mM) were without benefit and, paradoxically, at the higher concentrations resulted in a complete and irreversible loss of activity after anoxia. However, high concentrations of Mg2+ (110mM) significantly improved recovery (mean 64%, p<0.05). We conclude that disorder regulation of Ca2+ homeostasis induced by anoxia plays a central role in producing irreversible dysfunction in the RON. Furthermore, redistribution of Ca2+ under these conditions may not utilize conventional channels, since known blocking agents conferred no increased resistance to anoxia.

Supported by NIH and VA grants; P. Boyer is a BVA fellow.

456.19


We are examining the voltage- and ligand-gated conductances of solitary neurons in primary cell cultures of dissociated neurons from antennal lobes (ALs -- the primary olfactory centers in the brain) of the moth Manduca sexta as part of our ongoing studies of mechanisms that underlie processing of olfactory information. There are two basic classes of neurons within the AL: local interneurons (LNs) and projection (output) neurons (PNs). In cultures derived from the medial group of AL neurons, consisting exclusively of PNs, we previously identified 6 different morphological types [Soc. Neurosci. Abst. 14:380 (1988)]. We have now extended our analysis to cultures derived from the lateral group of AL neurons that contain both PNs and LNs. Along with the previously reported PN cell types, we have found 3 new types of neurons in these cultures. Cells of 2 of these new types clearly lack an axonal process. These neurons exhibit vigorous process outgrowth, but our patch-clamp studies have revealed that they fail to develop voltage-gated inward currents after 2 weeks in vitro. In contrast, all PNs develop inward currents after a comparable time in culture. We have also extended our analysis of Na+ currents. A subset of PNs expresses tetrodotoxin-sensitive Na+ channels. This current activates at about -40 mV and is fully activated at -20 mV, playing an important role in spike frequency adaptation. The time dependence of INa has been reported to be adequately accounted for by a two state kinetic model where the rates of channel opening and closing are voltage sensitive to an equal degree. (Adams et al., 1982, J. Physiol., 330, 537) That model predicts significant activation of INa by individual action potentials. We have found, however, that brief action potential-like voltage clamp commands activate much less INa than predicted. We recorded INa in acutely dissociated ganglion neurons using whole cell recording and discontinuous single electrode voltage clamp. Under conditions where currents other than INa were suppressed, INa was defined as the difference between currents recorded in the presence and absence of muscarinic. This allowed us to study INa kinetics over a wide range of membrane potentials. We found significant delays in INa activation lasting 4-10 ms depending on command potential. These delays are suggestive of multiple closed-states and ensure that INa depends on mean membrane potential rather than action potential frequency.

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THE KINETICS OF M-CURRENTS (I M) recorded in bullfrog sympathetic ganglion neurons. M.E.M. KELLY and P.S. PENNETHAM. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.

In bullfrog sympathetic cells inhibition of a voltage-dependent K+ current, I,M, by muscarinic agonists results in depolarization. I,M begins to activate at ~50 mV and is fully activated at ~20 mV, playing an important role in spike frequency adaptation. The time dependence of I,M has been reported to be adequately accounted for by a two state kinetic model where the rates of channel opening and closing are voltage sensitive to an equal degree. (Adams et al., 1982, J. Physiol., 330, 537) That model predicts significant activation of I,M by individual action potentials. We have found, however, that brief action potential-like voltage clamp commands activate much less I,M than predicted. We recorded I,M in acutely dissociated ganglion neurons using whole cell recording and discontinuous single electrode voltage clamp. Under conditions where currents other than I,M were suppressed, I,M was defined as the difference between currents recorded in the presence and absence of muscarinic. This allowed us to study I,M kinetics over a wide range of membrane potentials. We found significant delays in I,M activation lasting 4-10 ms depending on command potential. These delays are suggestive of multiple closed-states and ensure that I,M depends on mean membrane potential rather than action potential frequency.

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WITHDRAWN

456.22

MEMBRANE IONIC CURRENTS IN IDENTIFIED NEURONS OF A JELLYFISH. J. Pronielskiak and A. N. Spencer. Dept. of Zoology, Univ. of Alberta, Edmonton, AB, CANADA, T6G 2E9 and Bamfield Marine Station, Bamfield, BC, CANADA, V0R 1B0.

Nervous systems first evolved in the Cnidaria. We have described several neuronal membrane ionic currents in a hydrozoan jellyfish, Polyorchis penicillatus to determine whether they differ substantially from those observed in higher phyla and in Protocoz. It is possible to isolate and culture a subset of large, identifiable motor neurons from Polyorchis. In vitro, 'whole-cell', current-clamp recordings show that these cells produce plateau action potentials of variable duration, as they do in vivo. Voltage-clamp data have revealed that at least five distinct currents are at play. A large (~5nA), rapidly activating, transient calcium current produced the upstroke of the action potential; this current is TTX sensitive. A small (500pA), rapidly activating, transient (~200ms) calcium current contributes to the rising phase, and together with a slow, sustained calcium current, underlies a slowly repolarizing plateau phase. A large (~10nA), sustained, potassium mediated delayed rectifier contributes to spike repolarization. Spike repolarization is also affected by a large (~5nA) but transient (~10ms) calcium current which inactivates at depolarized voltages, thus regulating action-potential duration in a voltage-dependent manner.

It appears that the complememt of membrane currents found in these primitive neurons resembles that seen in 'higher' phyla, and differs in quality, but not in diversity, from that observed in Paramaecium.
456.23 VOLTAGE CLAMP ANALYSIS OF IONIC CONDUCTANCES IN TWO TYPES OF SENSORY NEURONS IN THE LEECH J. Johansen and A.I. Klembatsch. Dept. of Zoology, Iowa State Univ., Ames, IA 50011 and Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06512.

With the ultimate goal of correlating the biological function of neurons with their biophysical properties we have begun a systematic analysis of the ionic conductances underlying excitation in the lateral nociceptive (Nj) and medial pressure (Pn) cells in the leech Macrostemnus. The two cells differ in their functional sensory modalities and in their excitable properties. Using two electrode voltage clamp techniques we find that both Nj and Pn possess an IA, IK and IC. The peak Na-conductance is for voltage steps from resting membrane potential (-40 mV) to 10 mV. At this membrane potential at 10 °C IQ3 activates with a time constant of 1.6 ms and inactivates with a time constant of 9 ms. The kinetics of IA is best described by a Hodgkin-Huxley equation of the form $\frac{dQ}{dt} = g_{Na}m^{3}h(I - V)$. The delayed rectifier current (IR) activates with time constants of 6 to 15 ms at 22 °C in response to membrane steps to between -15 mV and 15 mV. This current was sensitive to TEA. ICQ does not inactivate appreciably during long voltage steps (>500 ms). The kinetic properties of these conductances were similar in both cells. However, in addition to these currents Nj possess an excitatory current with rapid activation and inactivation characteristics. In contrast, we have not yet been able to identify an IR-current in the Pn cell suggesting a possible mechanism for the differences in the two cell’s excitability. This possibility and a further characterization of the two cell’s conductances are currently being investigated.

456.24 WHOLE CELL PATCH CLAMP RECORDINGS OF VOLTAGE-DEPENDENT CURRENTS IN THIN MEDULLA SLICE INCLUDING IDENTIFIED TRIGEMINOPTHALAMIC NEURONS. L Chen and L-T.-M. Huang. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

In order to characterize the synaptic transmission to the trigeminothalamic neurons, we examined the electrical properties of neurons in caudal spinal trigeminal nucleus. Trigeminothalamic neurons were labeled with retrograde neuronal marker fluorescent latex microspheres. Thin slices were prepared from the lower medulla of the injected rats using the method described by Edwards, Konnerth, and Takahashi (Pflugers Arch, in press). The surface of the recording cells was blown clean with buffer, and whole cell recordings were then performed. Under voltage clamp conditions, both inward and outward currents were activated when a series of depolarizing pulses ranging from -60 to +150 mV were applied to the cell. Inward currents were examined by replacing $K^+$ with $Na^+$ or $Mg^2+$ and glutamate intracellularly, and by treating cells with tetrodotoxin (TTX) and 4-aminoopyridine. Most inward current could be eliminated by low concentration of tetrodoxin (TTX). $Na^+$ was the principal ion carrier of the inward current. In the presence of 1 µM TTX and 5-10 mM Ca, slow inward current was observed. This current was sensitive to external Ca concentration and was abolished by Co²⁺. Voltage-dependent outward currents were carried by $K^+$ and were blockable by TEA. The voltage properties of these currents were similar to those observed in isolated dorsal horn projection neurons (J. Physiol. 411:161-177, 1989). Supported by NS23065 and NS01050.


We report the first in vitro demonstration of functional expression of vertebrate taste receptor molecules in Xenopus oocytes. Injection of cRNA encoding the molecules responsible for the primary binding events in vertebrate chemosensation is dependent on functional in vitro assays encompassing the full range of transductive events from the initial ligand binding through changes in gated membrane conductances. Expression in the Xenopus oocyte has been used to identify, functionally characterize, and isolate cDNA encoding those in mammalian systems generally use separate optical paths for excitation and detection of the fluorescent signal or employ a microsphere. Thin slices were prepared from the lower medulla of the injected rats using the method described by Edwards, Konnerth, and Takahashi (Pflugers Arch, in press). The surface of the recording cells was blown clean with buffer, and whole cell recordings were then performed. Under voltage clamp conditions, both inward and outward currents were activated when a series of depolarizing pulses ranging from -60 to +150 mV were applied to the cell. Inward currents were examined by replacing $K^+$ with $Na^+$ or $Mg^2+$ and glutamate intracellularly, and by treating cells with tetrodotoxin (TTX) and 4-aminoopyridine. Most inward current could be eliminated by low concentration of tetrodoxin (TTX). $Na^+$ was the principal ion carrier of the inward current. In the presence of 1 µM TTX and 5-10 mM Ca, slow inward current was observed. This current was sensitive to external Ca concentration and was abolished by Co²⁺. Voltage-dependent outward currents were carried by $K^+$ and were blockable by TEA. The voltage properties of these currents were similar to those observed in isolated dorsal horn projection neurons (J. Physiol. 411:161-177, 1989). Supported by NS23065 and NS01050.


The two cells differ in their functional sensory modalities and in their excitability properties. Using two electrode voltage clamp techniques we find that both Nj and Pn possess an IA, IK and IC. The peak Na-conductance is for voltage steps from resting membrane potential (-40 mV) to 10 mV. At this membrane potential at 10 °C IQ3 activates with a time constant of 1.6 ms and inactivates with a time constant of 9 ms. The kinetics of IA is best described by a Hodgkin-Huxley equation of the form $\frac{dQ}{dt} = g_{Na}m^{3}h(I - V)$. The delayed rectifier current (IR) activates with time constants of 6 to 15 ms at 22 °C in response to membrane steps to between -15 mV and 15 mV. This current was sensitive to TEA. ICQ does not inactivate appreciably during long voltage steps (>500 ms). The kinetic properties of these conductances were similar in both cells. However, in addition to these currents Nj possess an excitatory current with rapid activation and inactivation characteristics. In contrast, we have not yet been able to identify an IR-current in the Pn cell suggesting a possible mechanism for the differences in the two cell’s excitability. This possibility and a further characterization of the two cell’s conductances are currently being investigated.

456.27 FLUORESCENCE RECORDING OF ACTION POTENTIALS USING A SINGLE OPTICAL FIBER CARRYING EXCITATION AND FLUORESCENT LIGHT. V. Gemmell, T. Busse, R. Davis, and R. Davis. Center for Devices and Radiological Health, Rockville, MD 20857, and USFMS, Dept. of Physiology, Bethesda, MD 20814.

Fluorescent dyes have been used previously to record voltage changes in a variety of preparations. Existing systems generally use separate optical paths for excitation and detection of the fluorescent signal or employ a microscope objective or other large lens system near the preparation which limits their use in confined areas. We used a single optical fiber to both excite and detect voltage-sensitive fluorescence signals from frog myocytes stained with W781. Light from a He-Ne laser, after passing through a neutral density filter and a beam splitter, was coupled to an optical fiber with an inner light-carrying core diameter of 4, 50, or 100 µ. The various fibers used had outside diameters of 120 µ due to optical clogging. The longer-wavelength fluorescent light from the preparation was collected by the same fiber and reflected, through a blocking filter, into either a photomultiplier. For photomultiplier currents, maintained in a 0 Ca²⁺ solution to prevent movement artifacts, produced a fractional change in fluorescence of $10^{-5}$ during the myocardial action potential. Large-core fibers yield quieter signals, which allowed for reduced excitation light intensity with less dye bleaching, but at the expense of the higher spatial resolution obtained with the single mode fiber.
This effect, coupled with the importance of the thalamic T current in reducing action of these agents might be mediated by the potential-sensitive fluorescent dye bis(2)-

DIFFERENTIAL EFFECTS OF ANTICONVULSANT AND CONVULSANT SUSTAINED (LM) CURRENT. MPS and ES reduced T current in a voltage-dependent manner. TMS blocked GABA responses in a concentration-dependent manner, whereas responses induced by TLA (10 μM) were unaffected by TLA treatment. The actions of ET and AVP dose-dependently increased DP. The actions of ET but not AVP were potentiated in the presence of BAY K 8644, whereas responses induced by AVP were not affected by treatment with either ET or AVP. These results suggest that extracellular Ca²⁺ channel conductance is enhanced predominantly by AVP in a G-protein-dependent manner and that Ca²⁺ channel conductance is enhanced presumably by a kinase phosphorylation of the Ca²⁺ channel itself or an associated regulatory protein. Supported by NSF Grant DCB812562. 457.6 COMPARISON BETWEEN THE ACTIONS OF EOSINOPHIL AND EOSINOPHIL IN PITHED RATS AFTER PRETREATMENT WITH BAY K 8644, NIFEDIPINE OR PERTUSSIS TOXIN. R. Tabriechi and C. B. Triggle. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

The pretreatment of endothenol and vasopressin in pithed rats after pretreatment with BAY K 8644, nifedipine or pertussis toxin. R. Tabriechi and C. B. Triggle. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.
LHRH AND GTP-γ-S MODIFY CALCIUM CURRENTS OF ISOLATED SLOWLY. Similar results have often been interpreted as selective dialysis with GTP-γ-S. Opening is delayed. Similar kinetics can be achieved by internal (see Figure). One possibility is that, at negative membrane potentials, only partially (~50%), and the remaining current activates more slowly than 1 μM irreversibly depressed the Δ[Ca2+]i. The concentration-dependent effects of ME on the concentration of met-enkephalin (ME, 1-30 nM) induced Δ[Ca2+]i may explain the finding that ME more effectively activates a decaying component of Ic2+-a (inactivated at a h.p. of ~50% (8-like)). In others, 5-HT caused a marked slowing of Ic2+- activation. This implies that 5-HT may alter the proportion of channels inactivated by current or voltage and hence the number capable of opening near the peak of the 1/V relationship. Supported by the Welcome Trust.

G PROTEIN-MEDIATED FMRF-AMIDERGIC MODULATION OF A CALCIUM CURRENT IN GIANT SYNAPSE. J.H. Maan, Son-Hing and P.G. Hayden, Department of Zoology, Iowa State University, Ames, IA 50011.

The neuropeptide, Phe-Met-Arg-Phe-NH2 (FMRFa) decreases the magnitude of a voltage-dependent calcium current and causes a presynaptic inhibition of transmitter release from cholinergic nerve fibers of Helisoma (Zoran et al., Neurosci. Abst. 1989; Hayden et al., Neurosci. Abst. 1989). In this study, we demonstrate that G protein mediates FMRFa's action on the calcium currents.

Various agents were included in the internal solution in the patch pipette during whole-cell recording from secretory somata. Four lines of evidence demonstrate G protein involvement in FMRFa's signal transduction pathway. 1) In the presence of either GTPγS (100μM; n=8) or GMP·P·NH2 (100μM; m=5), FMRFa caused an irreversible decrease in the calcium current. 2) GDP·S (100μM) completely prevented FMRFa's action. 3) AFa caused a rapid decline in the magnitude of the calcium current in the absence of FMRFa (n=8) and subsequent FMRFa application had no effect on the calcium current. 4) Addition of pertussis toxin prevented FMRFa's inhibition of the calcium current (n=6). These data demonstrate that a pertussis toxin-sensitive G protein is involved in mediating the inhibition of a calcium current by FMRF-amide.

Supported by a grant from the NIH, NS26650.

CALCIUM CHANNELS: MODULATION AND REGULATION THURSDAY PM

DEPHOSPHORYLATION OF VOLTAGE- Dephosphorylation of voltage-dependent calcium channel by purified protein phosphatases. Y. La and W. A. Catterall (SPON, B.M. Curtis). Department of Pharmacology, University of Washington, Seattle, WA 98195.

The Ca++ currents mediated by L-type voltage-dependent calcium channels (CaCh) have been shown to be modulated by protein phosphorylation in a variety of cells. The CaCh purified from skeletal muscle contains five specifically associated subunits. Two of the subunits, α2 and β, with Mr = 170,000 and β with Mr = 55,000, are phosphorylated by CaM-dependent protein kinase. Sarcoplasmic Ca++ phosphatization of these subunits activates purified CaCh reconstituted in phospholipid vesicles (Nunoki et al., Proc. Natl. Acad. Sci. USA, in press). Because of the importance of regulation of channel function by protein phosphorylation, we have studied the dephosphorylation of CaCh by purified protein phosphatases.

Purified CaCh was first phosphorylated by CaM-dependent protein kinase and then incubated with purified protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), or calcineurin (CN). PP1 and PP2A dephosphorylated both α2 and β subunits at rates comparable to those for the dephosphorylation of α2, a physiological substrate of both protein phosphatases. Similar results were obtained when CaCh reconstituted in phospholipid vesicles or CaCh in skeletal muscle transverse tubules were used as substrates. Recent results suggest that CN dephosphorylates β subunits of CaCh more rapidly than α2 subunits, but both rates are slower than those of PP1 and PP2A. We are currently examining the sites on α2 and β subunits that are dephosphorylated by the different protein phosphatases.


Chicken II luteinizing-hormone releasing hormone (LHRH) reduces whole-cell calcium current in frog sympathetic neurons, but only partially (~50%), and the remaining current activates more slowly. Similar results have often been interpreted as selective inactivation of a rapidly inactivating calcium channel. We suggest instead that the kinetics of a single type of channel are modified, since (i) the kinetics become more complex in LHRH, not less, as activation is fit by the sum of two exponential processes rather than one, and (ii) the calcium-phosphorylating property is lost reversibly (see Figure). One possibility is that, at negative membrane potentials, LHRH shifts a fraction of the channels into a state from which opening is delayed. Similar kinetics can be achieved by internal dialysis with GTPγ-S.

Figure. Effect of 100 nM LHRH on whole-cell current (2mM Ba2+). Note slow kinetics and rapidly desensitizing response.


Fura-2 measurement of [Ca2+]i was made simultaneously with intracellular recordings from single neurons. Submucousplexus preparations were made from the isolated caecum of the adult guinea-pig, sacculated by heavy stunning and bleeding from the neck.

Action potentials elicited a transient increase in intracellular [Ca2+]i, which was augmented by the concentration of met-enkephalin (ME, 1-30 nM) induced a small membrane hyperpolarization, a [Ca2+]i increase and a [Ca2+]i increase at 100 - 300 nM hyperpolarized the membrane, decreased the [Ca2+]i and attenuated the Δ[Ca2+]i. When the hyperpolarization was nullified, the [Ca2+]i increased and the attenuation of the Δ[Ca2+]i was abolished. ME more than 1 μM irreversibly depressed the Δ[Ca2+]i. The concentration-dependent effects of ME on the Δ[Ca2+]i may explain the findings that ME facilitates the sympathetic ganglionic transmission at low doses but inhibits it at high doses.
ENHANCEMENT OF RAT BRAIN CALCIUM CHANNEL ACTIVITY IN XENOPUS OCYCTES BY A PROTEIN KINASE C ACTIVATOR. Bradford E. Stiles, L. Yousif,1 and S. Christakos.* 1Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611 and 2Dept. of Biochemistry, New Jersey Medical School, Newark, NJ 07103.

Calcium currents were analyzed in cerebellar Purkinje cell bodies (PCs) acutely isolated from rats between postnatal days 7-13 following pharmacological treatment (cf. Regan, L. Soc. Neurosci. Abstr. #31.7, 1987). Isolated PCs were identified using immunocytochemical staining for the PC-specific, calcium-dependent chloride current. Whole-cell recordings were performed as described elsewhere (Miller, N. Nerve 107: 275-280, 1984). Currents ranging from 200 to 1000 nA. The effect of 100 nM forskolin was examined at this stage of development (n=64). Low threshold (T-type) calcium currents were observed using two-electrode voltage-clamp. The effect of Ca2+ currents were not affected by forskolin at either 1 sec or 2 sec after a command to +10 mV. Maximal block was achieved at the peak of the voltage/current curve (+10 to +15 mV). In addition, forskolin increased the apparent slowing of the rising phase of the current at potentials positive to +10 mV. If similar events occur at adrenergic nerve terminals, these results may explain the presynaptic inhibition of action of forskolin (Supported in part by the Pharmaceutical Manufacturers Association Foundation).
LOCALIZATION OF CALCIUM INFLUX THROUGH PHORBOL ESTER-INDUCED CALCIUM CHANNELS. L.A. Fink*, L.K. Kaczmarek, and J.A. Connor. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510; Roche Institute of Molecular Biology, Nutley, NJ 07110

Activators of protein kinase C have been shown to enhance the calcium current in the bag cell neurons of Aplysia by unmasking a 24 pS voltage-dependent calcium channel in the plasma membrane. This species of channel is not observed in control cells in which the kinase has not been stimulated, although control cells possess calcium channels of lower unitary conductance (~12 pS). We have now used fluorescent imaging of the calcium-indicator fura-2 to compare the localization of calcium influx in control cells and in cells exposed to the protein kinase C activator, TPA (20 nM). Single bag cell neurons with extensive neuritic outgrowth after one day in culture were loaded with fura-2 through a microelectrode, and fluorescent ratio images were generated with a cooled CCD-based imaging system. In control cells, a train of action potentials generated large, transient calcium signals in the calcium signal from the neurites, with little or no increase in calcium at the soma. In contrast, after exposure of the cells to TPA for 10-15 min, much larger elevations of calcium at the soma were observed in response to action potentials. The depolarization-induced elevation of calcium in the neurites was also enhanced by TPA. Our data suggest that the large conductance calcium channels induced by activators of protein kinase C are located both on the soma and the neurites of isolated bag cell neurons, whereas, calcium influx through the lower conductance channels in both control and activated cells occurs primarily in the neurites.

WITHDRAWN

CALCIUM CHANNELS: MODULATION AND REGULATION THURSDAY PM

LIGAND-GATED ION CHANNELS II

THE BENZODIAZEPINE DIAZEPAM AND THE β-CARBOLINE DMCM MODULATE SINGLE CHANNEL CURRENTS BY OPPOSITE MECHANISMS. C.J. Rogers, P.E. Twyman and R.L. Macdonald. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104

Diazepam (DZ) and DMCM bind to the benzodiazepine binding site of the GABA<sub>A</sub> receptor channel and increase and decrease GABA receptor current, respectively. The effects of DZ and DMCM on single channel GABA<sub>A</sub> receptor currents were studied to determine their mechanisms of action. Outside-out patches from mouse spinal cord neurons grown in cell culture were held at -75 mV in symmetric chloride solutions. Data were digitized at 20 kHz with 2 kHz Bessel filtering. Data from different patches were pooled respectively. The effects of DZ and DMCM on single channel MODULATE Dept, of Neurology, Univ. of Michigan, Ann Arbor, MI 48104.

GABA receptor single channel currents in a concentration dependent manner without altering mean open time. Open durations of GABA<sub>A</sub> with similar relative frequencies of occurrence. GABA<sub>A</sub> receptor currents occurred in bursts of openings (separated by closures greater than 5 to 150 msec). Burst and cluster frequencies were increased and decreased by DZ and DMCM, respectively. These data suggest that the major effect of DZ is to increase the affinity of GABA for the GABA<sub>A</sub> receptor, an effect opposite to that of DMCM which predominantly decreases the affinity.

CYCLIC AMP DEPENDENT PROTEIN KINASE DECREASES GABA<sub>A</sub> RECEPTOR CURRENT IN MOUSE SPINAL NEURONS. N.M. Porter*, R.E. Twyman, M.D. Uhler*, and R.L. Macdonald. Dept. of Neurology and the Mental Health Research Institute*, Univ. of Michigan, Ann Arbor, MI 48104

The predicted structure of the neuronal bovine GABA<sub>A</sub> receptor places a consensus phosphorylation site for cAMP dependent protein kinase (PKA) on an intracellular domain of the receptor. To determine the role of PKA-mediated phosphorylation on GABA<sub>A</sub> receptor function, we measured GABA receptor chloride currents in the presence of PKA in mouse spinal neurons using the whole cell and patch clamp techniques. The bath medium contained (mM): 142 NaCl, 8.1 KCl, 1 CaCl<sub>2</sub>, 6 MgCl<sub>2</sub>, 10 glucose, and 10 HEPES. In whole cell recordings, application of GABA (5 µM) to the soma reproducibly evoked an inward chloride current and an increase in membrane conductance. Inclusion of the purified catalytic subunit of PKA (50 µM) in the pipette solution reduced the GABA receptor current by 59% (n = 35). The GABA receptor current was not reduced, however, in cells in which heat-inactivated catalytic subunit was substituted for the active subunit in the pipette solution (n = 8). Application of PKA to excised, outside-out patches evoked excitatory chloride currents in control patches (n = 10). In PKA patches very few openings were recorded and the total GABA receptor current was reduced by 97% (n = 7). PKA decreased GABA receptor current primarily by reducing the frequency of channel opening. These results suggest that phosphorylation of the GABA<sub>A</sub> receptor, or of a protein associated with the channel, regulates chloride channel opening. Supported by NIH NS058216 to NMP and DA04122 to RLM.
458.3

To determine gating kinetics of the GABAA receptor channel, intraburst properties of the main conductance state (27 ps) were investigated with the outside-out patch clamp recording technique. Intraburst openings obtained from mouse spinal cord neurons grown in cell culture were held at -75 mV in symmetric chloride solutions. Data were digitized at 8 kHz with a 1 kHz Bessel filtered current. A frequency distribution of openings per burst, intraburst openings, individual openings, successional openings and total open time of bursts containing 1 to 5 openings were analyzed by curve fitting to various models or gamma functions. Intraburst closed times were analyzed similarly.

The analysis confirmed the presence of 3 concentration indep­endent open states (durations of 1.0, 2.9 and 8.1 ms) and 2 intraburst closed states (0.2 and 2.0 ms). The results suggested that the GABA receptor main conductance state opens into at least one brief open state from a singly liganded closed state and two longer duration open states from doubly liganded closed states. Channel openings were grouped into bursts with three different mean durations. Bursts were formed primarily by repeated openings of individual open states. The shortest open state opened on average 1.1 times, while the medium- and longest duration open states opened on average 2.0 and 3.9 times, respectively. The brief intraburst closures may represent open channel blocked states or channel closed states which are independent of ligand associated gating. Each open state bursts, therefore, due to channel blocking or intrinsic channel closure rather than due to ligand associated channel gating. A kinetic model consistent with these observations will be presented.

458.4

Alpha-cyano pyrethroid insecticides have been shown to interact with the GABA(A) receptor-ion channel in a number of different ways. We have found that the acute application (c 2 min) of the cyano pyrethroids fenvalerate and dieldrin (HE) at concentrations up to 50 µM can enhance (or suppress as one might expect) GABA-activated chloride currents in cultured hippocampal (HC) neurons (Frey et al., Toxicology Letters, 9:149, 1989). In an effort to understand this phenomenon, we tested the effects of 5-HT (1-100 µM) on pentobarbital (PB)-induced chloride currents in HC neurons recorded in the whole cell mode. Application of PB (100-300 µM) by puff for 20-30 sec elicited inward currents (I(Inward) > I(Outward) which reversed at the Cl equilibrium potential and were blocked by both picrotoxin and benzodiazepine (BDZ) at concentrations <40% over 9-12 min. The S component was increased by 5-HT concentration. Both T and S components were reduced reversibly with a relative potency of endrin = isobenzan > HEOD = HE.

Lindane blocks GABA-activated CI currents in cultured hippocampal neurons (Ogata et al., PNAS, 72:2896, 1985; Frey et al., Toxicology Letters, 28:141, 1987). We have compared the effects of lindane and the cyclodienes in cultured rat hippocampal neurons using the whole cell patch clamp technique. GABA (13 µM) plus 5-HT (100 µM) delivered by puffer for 20 sec, produced rapid inward currents with distinct early transient (T) and late sustained (S) components (see Dichter and Frey, this meeting). Both T and S components were reduced reversibly (50 and 304, respectively, 10 µM) by either coapplication or a single 2 min preapplication of lindane. The cyclodienes dieldrin (HEOD) and isobenzan (1-50 µM) also reduced GABA/Cl currents with a relative potency of endrin = isobenzan > HEOD = HE. However, the onset of action of the cyclodienes was much slower than lindane. Continuous application of HE or HEOD at 10 µM blocked 50% of the T component in -6 min, and the current continued to decrease to <40% over 2-12 min. The S component was reduced by <25% within 7 min but remained unchanged with subsequent application. The effects on both components were irreversible. Thus, both lindane and the cyclodienes blocked GABA/Cl currents although their time course of action were markedly different. The results are compatible with the convulsant action of these insecticides. Supported by NIH grant NS14143.

458.5

Lindane blocks GABA-activated Cl- currents in cultured neurons (Ogata et al., PNAS, 72:2896, 1985; Frey et al., Toxicology Letters, 28:141, 1987). We have compared the effects of lindane and the cyclodienes in cultured rat hippocampal neurons using the whole cell patch clamp technique. GABA (13 µM) delivered by puffer for 20 sec, produced rapid inward currents with distinct early transient (T) and late sustained (S) components (see Dichter and Frey, this meeting). Both T and S components were reduced reversibly (50 and 304, respectively, 10 µM) by either coapplication or a single 2 min preapplication of lindane. The cyclodienes dieldrin (HEOD) and isobenzan (1-50 µM) also reduced GABA/Cl currents with a relative potency of endrin = isobenzan > HEOD = HE. However, the onset of action of the cyclodienes was much slower than lindane. Continuous application of HE or HEOD at 10 µM blocked 50% of the T component in -6 min, and the current continued to decrease to <40% over 2-12 min. The S component was reduced by <25% within 7 min but remained unchanged with subsequent application. The effects on both components were irreversible. Thus, both lindane and the cyclodienes blocked GABA/Cl currents although their time course of action were markedly different. The results are compatible with the convulsant action of these insecticides. Supported by NIH grant NS14143.

458.7
SEROTONIN DIRECTLY EXCITES SPINAL MOTOEURONS. A.J. Berger and T. Takahashi. Dept. of Physiol., Faculty of Medicine, Kyoto Univ., Kyoto, Japan.

Serotonin (5-HT) increases excitability of spinal motoneurons (Ms) (Barasi et al., Br. J. Pharmacol. 52:339, 1974), but the mechanism for this effect is unknown. We studied this by applying whole-cell recording to neonatal rat Ms visually identified in thin spinal cord slices. In current clamp mode, bath-applied 5-HT (10 µM) in nominally Ca-free Krebs (5 mM K+ Krebs) solution depolarized Ms beyond firing threshold. In voltage clamp, at approx. -50 mV in Ca-free Krebs containing TTX, 5-HT generated an inward current (I(Inward)) which associated with a conductance increase. External application of Cs (10 mM) but not Ba (2 mM) virtually abolished the I(Inward). At higher external K concentrations (30 mM), peak amplitude of the I(Inward) was larger. The I(Inward) current-voltage relation showed inward rectification. In 20 mM K+, the I(Inward) reversal potential was -97 mV. These I(Inward) characteristics were similar to those of the inward rectifying current seen in these Ms during membrane hyperpolarization. We conclude that 5-HT directly excites spinal motoneurons and this is probably mediated by the inward rectifying current present in Ms.

(Supported by Grant No. 63641515 from the Ministry of Education, Science and Culture of Japan to TT, and a USPHS Fogarty Senior Fellowship to AJB)
458.10

A PROTON-ACTIVATED SUSTAINED INWARD CURRENT IN RAT DORSAL ROOT GANGLION NEURONS. S. Bray* and J.C. Yearg (SPON: Brain Research Association), Sandison Institute for Medical Research, 3 Goslar Street, London WC1E 6BN, UK.

A reduction in the external pH of the medium bathing rat and chick DRG neurons to pH 6.4 elicits a transient inward current, which relaxes in 1-2 s (Kristal & Hudspeth, 1980; Komemt et al., 1987). We have found that lowering the pH to <6.4 also activates a sustained inward current in a sub-population of DRG neurons.

3-7 day old cultures of neonatal rat DRG neurons responded to acidification of the external medium to pH 6.4 with an influx of 14C-guanidinium and 3Hborate. This influx was abolished when cultures were pretreated for 24 hours with 2-10µM phenytoin, a calcium channel blocker. We have found that lowering the pH to <6.4 also activates a sustained inward current in a sub-population of DRG neurons.

We conclude that phenytoin may be acting in a site distinct from the GABA-benzodiazepine receptor complex to potentiate GABA-mediated chloride uptake.

458.14

GABA AND BENZODIAZEPINE RECEPTORS IV

459.1


We have previously demonstrated that diazepam potentiated the chloride current in 3H-guanidinium in rat brain (Okazaki, M. et al. Life Sci., 33: 409, 1983). The current study attempts to investigate, conversely, whether phenytoin interacts with the GABA receptor complex or any of its subunits.

GABA-activated chloride flux in a synaptosomal preparation from rat cerebral cortex was used. GABA (10 µM) stimulated chloride uptake into synaptosomes by 47% over basal flux. Diazepam (50 µM) had no effect on its own, but potentiated GABA-stimulated chloride flux by 25%. Phenytoin (10 µM) had no effect on basal or GABA-stimulated chloride flux.

However, phenytoin significantly enhanced chloride uptake by 18% (p<0.05) (n=4).

Taken together, these data suggest the possibility that, in mammalian brain, the phenytoin recognition site may be allosterically coupled to a benzo diazepine recognition site. SiteB and, in turn, may be functionally linked with the GABA-A receptor complex.

459.2


Endogenous metabolites of progesterone and deoxycorticosterone potentiate GABA-mediated chloride ion flux in brain synaptosomes. The 3α-hydroxy-5α-androstane-17ß-carbonitrile concentration response curves were generated for steroid hormone metabolites of progesterone and deoxycorticosterone in brain synaptosomes. The 3α-hydroxy-5α-androstane-17ß-carbonitrile concentration response curves were generated for steroid hormone metabolites of progesterone and deoxycorticosterone in brain synaptosomes. The 3α-hydroxy-5α-androstane-17ß-carbonitrile concentration response curves were generated for steroid hormone metabolites of progesterone and deoxycorticosterone in brain synaptosomes.
CHRONIC LITHIUM EFFECT ON GABA-MEDIATED SYNAPTONEUROSOMAL CHLORIDE UPTAKE. P.A. Chmielewski and J.A. Miller (SPON: R.J. Dinerstein), Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT 06032.

We have been investigating the role of γ-amino butyric acid (GABA) receptors in the biological activity of chronic lithium in a CNS using a synaptoneurosome preparation, the functional response following stimulation of the GABA-BZD-chloride channel complex. In this preparation the uptake of 36Cl⁻ in the second exposure. Cerebral cortical synaptoneurosmes from rats fed a lithium-containing (40 mMol) diet for 3 weeks are responsive to muscimol stimulation. (Serum Li levels for these animals were 0.90 ± 0.05 µM.) At 20 µM muscimol, uptake stimulation increased (from control 166% above baseline to 236%) representing a 107% increase in muscimol stimulation (p<0.05). Acute Li exposure over a wide physiological range has no effect on muscimol stimulated chloride flux but at supraphysiologic concentrations of lithium inhibition of Cl⁻ uptake was observed. This may suggest that a chronic lithium effect in man may be mediated in part by functionally altering GABA-mediated chloride flux. Another observed effect was an enhancement of chloride uptake in the absence of magnesium, which is not reversed by lithium. This work was partially supported by the University of Connecticut Health Center Research Advisory Committee.

PYRETHROID INSECTICIDES AND VERATRINE INCREASE BASAL CHLORIDE UPTAKE INTO RAINBOW TROUT SYNAPTONEUROSOMES. A.J. Eshleman* and T.F. Murray. Toxicology Program and College of Pharmacy, Indiana University School of Medicine, School of Veterinary Medicine, and Veterinary Research Institute, College of Agriculture, Purdue University, 47907-1228.

The radioligand [3H]-flunitrazepam ([3H]-FLU) which binds to high affinity GABA-A receptors and decreases the ATP effects are also observed with GTP but not with non-hydrolysable ATP's (GTPγS). The effect of the ATP effects are not duplicated by 8-bromo-cAMP or the hydrolyzable nucleotides. These suggestions are consistent with the idea that phosphorylation increases GABA receptor desensitization. Of the SCI in the third state. Thus, the reduction of the maximal GABA response corresponds to an enhancement of GABA receptor desensitization.

THE INHIBITION OF [3H]-FLUNITRAZEPAM BINDING IN WASHED RAT BRAIN MEMBRANES AND BRAIN TISSUE SECTIONS BY ß-CARBOLINES. P.A. Chmielewski and T.F. Murray. Toxicology Program and College of Pharmacy, Indiana University School of Medicine, School of Veterinary Medicine, and Veterinary Research Institute, College of Agriculture, Purdue University, 47907-1228.

The chloride influx: relationship between GABA uptake rate and GABA concentration. I. Acetylcholine. J. A. Miller*. Richard D. White, Steven C. Blier. Department of Psychiatry, Neurosciences Program, The Ohio State University, Columbus, OH 43210-1228.

GABA produces a concentration dependent increase of 36Cl⁻ uptake in membrane vesicles prepared from rat cerebral cortex. The transformation of these data as specific chloride influx ([IC₅₀] of GABA) as a function of 36Cl⁻ flux shows a concentration of 10 µM GABA. Second exposure of the GABA receptor antagonist R015-1788 by a rapid increase in the SCI over a GABA concentration range of 0-10 µM and reaches a maximum at 10 µM GABA. The second exposure state is defined by the SCI value which occurs between 10-30 µM GABA. The third exposure state occurs at GABA concentrations over 30 µM, shows declining values of SCI, and is probably related to the desensitization of the GABA receptor. A fixed concentration of 100µM of the ß-carbolinic agonist for the BZ-receptor, ZK 93423, shifts to the left the GABA dose-response curve at lower GABA concentrations while depresses the maximal GABA response at higher GABA concentrations. The effects of ZK 93423 on SCI are (1) an increase in SCI value in the first and second exposure state, and (2) a decrease in SCI in the second state from steady to very fast declining SCI; (3) a decrease of SCI in the third state. Thus, the reduction of the maximal GABA response corresponds to an enhancement of GABA receptor desensitization.
459.9
BUSHFIRE ALTERS BENZODIAZEPINE BINDING BUT NOT GABAa RECEPTOR FUNCTION. F. Lopez,* L.G. Miller. A. Medical Center, Durham, NC 27710.
Rochelle D. Schwartz. Dept. of Pharmacology, Duke University
Scatchard analysis bound to the molecular vs granular layer of the cerebellum.
CHARACTERIZATION AND DISTRIBUTION OF [35S]TBPS BINDING TO RAT
preincubated slices markedly but variably decreased [35S]TBPS binding in vivo and ex vivo. [35S]TBPS binding in vitro was not affected by B and IPP, but number of sites was increased by G, 5 mg/kg. Muscimol-stimulated chloride uptake was unaffected by B, and IPP, but number of sites was increased by G, 5 mg/kg. Muscimol-stimulated chloride uptake was unaffected by B, G or IPP (Vehicle 25.8: B 25.6; G 25.0; IPP 25.7; all µmol/mg proc). Thus, buspiron and gepirone may affect EZ binding but do not appear to alter function of the GABAa receptor.
The metabolite IPP does not affect binding or receptor function.

459.10
QUANTITATION OF CELL-SURFACE VS. INTRACELLULAR BENZODIAZEPINE RECEPTORS USING A SULFONATED BENZODIAZEPINE DERIVATIVE. M.H. Jaliian Tehrani and E.M. Barnes, Jr. Dept. of Biochemistry, Baylor College of Medicine, Houston, TX 77030.
A membrane-impermeant derivative of the benzodiazepine 1012S was prepared by reaction of 1012S with 3-hydroxy- 
fusolysinophosphonic acid and purification by reverse phase HPLC. The resulting N-[4-sulfophenyl)methyl] derivative retained the high affinity of the parent compound for central benzodiazepine receptors on chlcor cortical neurons in culture. Approximate 50% displacement of [3H]flunitrazepam binding to intact neurons were 2 nM and 5 nM for SPTC-1012S and 1012S, respectively. Permeabilization of neuronal surface membranes by exposure of cells to 0.005% saponin had no effect on the level of [3H]flunitrazepam binding or on the ability of 1012S to displace this label. However, [3H]flunitrazepam displacement by SPTC-1012S was increased by 10% following saponin treatment. The data suggest that 90% of cellular benzodiazepine receptors are exposed on the external surfaces of developing neurons. Thus, SPTC-1012S can be used to study the intracellular trafficking of GABA/benzodiazepine receptors.
Supported by NIH grants DK 17436 and NS 11535.

459.11
[35S]TBPS BINDING EX VIVO AS AN INDEX OF IN-VIVO GABAERGIC ACTIVITY AND MODULATION. E. Concato,* E. Sanna*, M. Serra* and S. Saling. Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.
The pharmacology of GABAa receptor was studied by measuring "ex vivo" [35S]TBPS binding in unwashed membrane preparations from rat cerebral cortex. Anxiogenic and convulsant 6-carboline derivatives produced a dose dependent (0.05-1 mg/kg i.v.) increase of [35S]TBPS binding. On the contrary, benzodiazepines (BZ) and other agonists for BZ receptors markedly decreased [35S]TBPS binding in the same brain area. The effect of anxiogenic and anxiolytic drugs was reversed by isoxazolidine (100 µg/kg i.p. i.v.), an inhibitor of GABAergic transmission, and valproic acid (150-400 mg/kg), respectively. No 15-1788, a BZ receptor antagonist, prevented the effects of both anxiogenic and anxiolytic drugs but failed to antagonize the action of isoxazolidine and valproic acid. Ethanol (1-4 gr/kg) like anxiolytics elicited in 35S-TBPS binding. The action of ethanol was also studied on both the convulsive pattern and the increase in 35S-TBPS binding elicited by isoxazolidine. High doses (3-5 gr/kg) of ethanol prevented the convulsions elicited by isoxazolidine. Consistently ethanol antagonized by 50% isoxazolidine-induced increase in [35S]TBPS binding. The results suggest that the "ex vivo" measurement of [35S]TBPS binding is a suitable index of the functional state of GABAa receptors in vivo.

459.12
Zolpidem (20), is a highly selective ligand for the w1 (BZ) site in the GABA receptor supramolecular complex (GRSC) (Langer, S. and Apollis, S. J. Fund Clin. Pharm. 2: 109, 1981), respectively. In order to determine if selective w1 site stimulation is sufficient to modulate the GRSC function, we have presently examined the action of 20 at different subunits of the GRSC using [3H]-TBPS binding and 36Cl-uptake. With well washed rat cerebral cortex membranes in the presence of NaCl, 20 allosterically enhances [35S]TBPS binding (EC50 100µM, Max. eff. +31%). This effect is competitively antagonized by flumazenil (1 and 10µM) but bicculline (10µM) only partially (33%) inhibits the action in a non-competitive manner. The action of 20 is completely antagonized by bicculline and unaltered by flumazenil. The enhancement of [35S]TBPS binding by 20 appears to be due to an enhanced rate of association of the ligand with the receptor. The action of 20 is also modulated (Introduction of a fast component of dissociation) suggesting that 20 enhances the frequency of chloride ionophore opening and closing. The findings using [35S]TBPS binding are confirmed by 36Cl-uptake studies. 20 increases [35S]TBPS binding to rat cerebral cortex membranes, apparently by enhancing the number of sites available to the ligand. Thus, selective w1 site activation by ligands such as 20 is capable of activating the entire sequence of events within the GRSC.

459.13
CHARACTERIZATION AND DISTRIBUTION OF [35S]TBPS BINDING TO RAT BRAIN SECTIONS USING AUTORADIOGRAPHY. Patricia F. Edgar and Rochelle D. Schwartz. Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.
[35S]TBPS binding to rat brain sections was characterized for subsequent autoradiographic analysis. Cortical brain areas were incubated with [35S]TBPS to remove endogenous GABA. The association and dissociation rate constants were 0.37 Min-1µM-1 and 0.011 min-1, resp. Dissociation was monophasic and represented by a single population of binding sites (Kd = 21.0 nM, Smax = 1.59 pmol/mg prot). Picrotoxin and muscimol inhibited [35S]TBPS binding with IC50 of 5 and 100 µM, respectively. Distribution of [35S]TBPS binding sites in the rat brain resembles that of other ligands used to identify GABAa receptors with some regionally specific differences. Regions with a high degree of [35S]TBPS binding include inf. colliculus, med. septal n., central thalamic n., olf. tubercle, zona incerta, dentate gyrus, and substantia nigra. These regions are strongly bound to the molecular vs granular layer of the cerebellum. Omission of the preincubation or addition of 1 µM GABA to preincubated slices markedly but variably decreased [35S]TBPS binding. For example, [35S]TBPS binding was inhibited to different degrees in the cell layers of the cerebellum. Our study shows that the distribution of [35S]TBPS binding sites is influenced by the preincubation-incubation conditions.
Supported by NIMH S 7M and NIA Foundation.

459.14
Monoclonal antibodies (bd-17 and bd-24) to the benzodiazepine/GABAa receptor complex purified from bovine cerebral cortex were used to study the localization of this receptor in immunohistochemical analysis. The patchwork analysis indicated a single population of binding sites (Kd = 21.0 nM, Smax = 1.59 pmol/mg prot). Picrotoxin and muscimol inhibited [35S]TBPS binding with IC50 of 50 and 30 µM, respectively. Distribution of [35S]TBPS binding sites in the rat brain resembles that of other ligands used to identify GABAa receptors with some regionally specific differences. Regions with a high degree of [35S]TBPS binding include inf. colliculus, med. septal n., central thalamic n., olf. tubercle, zona incerta, dentate gyrus, and substantia nigra. These regions are strongly bound to the molecular vs granular layer of the cerebellum. Omission of the preincubation or addition of 1 µM GABA to preincubated slices markedly but variably decreased [35S]TBPS binding. For example, [35S]TBPS binding was inhibited to different degrees in the cell layers of the cerebellum. Our study shows that the distribution of [35S]TBPS binding sites is influenced by the preincubation-incubation conditions.
Supported by NIMH S 7M and NIA Foundation.
459.15

AUTORADIOGRAPHIC LOCALIZATION OF GABA AND GABAï¿½ BINDING SITES IN THE VENTRAL HORN OF THE CAT SPINAL CORD. Detra A. Vascil, Joseph P. Hoffman, Jr. and Bruce P. Malenka. Dept. of Anatomy and Neurobiology and Dept. of Pharmacology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

Gamma-aminobutyric acid (GABA) is considered to be a major inhibitory neurotransmitter in the central nervous system. Our previous studies have demonstrated a uniform distribution of GABA immunoreactive synaptic terminals in the ventral horn of the cat. In the present study we have examined the presence of GABA and GABAï¿½ binding sites in cat spinal cord levels C4-L6 using 112H-muscimol to label high affinity GABA binding sites and 11H-baclofen for GABAï¿½ binding sites. Each ligand was used at a concentration at or below its calculated Kd. Autoradiograms of 11H-muscimol labeled tissue sections demonstrated moderate grain density in a uniform pattern over the entire ventral horn. Although 11H-baclofen labeled tissue sections had an overall lower grain density than GABA binding sites, its binding sites were present in the same regions of the ventral horn as the GABA binding sites. The distribution of GABA and GABAï¿½ binding sites in a pattern similar to GABAergic immunoreactive terminals, suggests a possible morphological correlate for GABAergic receptors in the ventral horn of the cat. Supported by NIH Grant NS 23861 to B.E.M.

459.17

AUTORADIOGRAPHIC COMPARISON OF PERIPHERAL-TYPE HISTAMINE AND DOPAMINE RECEPTORS. L. P. Schoultz, Jr.; T. A. Miller* and R. W. Olsen. Dept. of Entomol., Univ. of Calif., Riverside, CA 92521.

Receptor autoradiography was used for the localization and characterization of CNS GABA receptors using GABA agonists and antagonists and competitive binding to 11H-muscimol and 11H-TIPS. Competitive binding results were compared to sections incubated in 11H-muscimol. Adjacent sections were utilized in the incubation procedure whereas the sections incubated in 11H-TIPS were dissected out, frozen, sectioned, incubated, dried and exposed to LKB Ultrafilm. Images were processed, evaluated and digitized using the autoradiograms via a linear photodiode array 12-bit CCD camera. Digitized images were transferred to an imaging analysis program where dimensional images of brain and thoracic ganglia were compiled from standard curve obtained from digitizing calibration standards. Three-dimensional images of brain and thoracic ganglia were prepared. The composite images were stored as a new image thus allowing direct readings of optical density from each autoradiographic image. Optical density readings were automatically converted to DPM based on a standard curve obtained from digitizing calibration standards. Three-dimensional images of brain and thoracic ganglia were compiled from concurrent adjacent sections and transferred into a three-dimensional reconstruction program. Our results indicate competitive binding of GABA receptors was seen for muscimol and TIPS. Overlay subtraction of images showed specific GABA receptor locations for the lamina ganglionaris, some portions of the medulla, lobula plate and glomerulus. GABA receptors were also localized in the thoracic ganglia in specific lateral regions.

MONAMINES AND BEHAVIOR IV

460.1

THE ACUTE DOPAMINERGIC ACTIVATING EFFECTS OF NICOTINE ARE REDUCED FOLLOWING REPEATED ADMINISTRATION. P. Vezina, G. Blanc, J. Glowinski and J.-P. Tassin. Chaire de Physiologie, Université de Louvain, Place Des Arts, BRUXELLES, BELGIUM.

Acute systemic injections of nicotine produce increased locomotion in rats and repeating these injections has been shown to produce a more pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the pronounced or enhanced locomotor effect.

459.16

AUTORADIOGRAPHIC COMPARISON OF PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITES IN HUMAN AND RAT KIDNEY. J.M. Olson, B.L. Cillins, W.R. Maroni and A.R. Young. Dept. of Pharmacology and Neurology and the Neuroscience Program, University of Michigan, Ann Arbor, MI 48170.

The affinity of isoxazolines, benzodiazepines and diuretics were compared autoradiographically in postmortem human and rat kidneys. No interspecies differences were observed in the binding of the isoxazoline PK 11195 (Human: Kd = 6.1 ± 1.5 nM, R2 = 0.53 ± 0.16 µg/mg prot.; Rat: Kd = 7.2 ± 0.3 nM, R2 = 0.75 ± 0.07 µg/mg prot.). The benzodiazepines Ro 45-4645, diazepam and flunitrazepam however, bound with lower affinity to human kidney (Kd = 117 ± 65 nM, R2 = 0.11 ± 0.06) than to rat kidney (Kd = 87 ± 12 µM, R2 = 0.70 ± 0.20 nM, R2 = 0.10 ± 0.34 M, respectively). Homologate studies confirmed the interspecies difference. In both species, the apparent affinity of benzodiazepines for PBRs was significantly higher in homogenate studies than in autoradiographic studies.

460.2


The role of dopaminergic neuronal systems in behavioral abnormalities of Lesch-Nyhan syndrome and some mental retardation was investigated by measuring the effects of DA and D2 DA agonists on supersensitive DA receptors in rats. Previous studies have shown that microinjections of amphetamine into the middle ventrolateral (MV) portion of the striatum produce locomotor stereotypy (A. Kelley et al., Psychopharmacology 95:356, 1988). We have now investigated the effects of microinjections of DA agonists into this striatal area of rats with supersensitive DA receptors produced by unilateral denervation of the nigrostriatal DA neurons with 6-OH-DA. Surgical procedures were carried out under pentobarbital anesthesia and recovery from surgery was monitored. The microinjections of the mixed D2/D3 agonists quinpirole (Ro 60-0175), 1 µg, and the D2 DA agonist quinpirole, 1 µg, were delivered into the MV portion of the striatum (coordinates A: 0.25, L: 1.0, V: 7.6) using a micromanipulator. BB induction was monitored by visual observation and recorded on a 1-minute interval for 1 hour post injection. No significant effects were observed with the above drugs.

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460.3

Psychostimulant drugs have been shown to enhance conditioned reinforcement. In this study, hungry rats were trained to associate a compound stimulus (light/C);click) with a food pellet. During the test phase, two levers were introduced, and pressing the correct lever (CR lever) was reinforced by the stimulus alone. On four test days, amphetamine (0.2, 1.0, 2.0, 20.0 μg) was infused into the following regions in separate animals: nucleus accumbens, ventromedial (VMS), ventrolateral (VLS), anterior dorsal (ADS), and posteral dorsal (PSD) striatum. Amphetamine infused into the nucleus accumbens enhanced conditioned reinforcement (CR). Infusion into the VMS selectively enhanced CR at the medium dose, but abolished responding at the higher doses. Infusion into the ADS significantly increased CR only at the highest dose. Infusion into the PDS had no significant effect. In further experiments, substance P (SP) and α-mela-enkephalin were injected into the lateral tegmental area (VTA). SP caused a small increase in lever pressing, but this effect was not selective for the CR lever. DAVA had no effect on lever pressing.

460.5

Administration of the neuroleptic drug metoclopramide (MET, 5.0 mg/kg) potentiated freezing responses of rats to 1.0 mA footshock, but did not produce any pre-shock freezing. To determine if inappropriate freezing responses could contribute to deficits in active avoidance produced by MET and other neuroleptics, drugged and undrugged rats received three one-way avoidance trials, each of ten one-way avoidance trials, in one experiment, or prior to the avoidance session, in a second experiment. In neither case was there evidence of control rats being adversely affected, but in each case the performance of MET-treated rats was significantly disrupted. A third experiment demonstrated that presentation of a conditional stimulus previously paired with shock disrupted the avoidance performance of MET-treated rats. We conclude that the enhancement of freezing by neuroleptic drugs contributes to the deficit in active avoidance produced by their administration.

460.6
HISTAMINE AND NEUROLEPTIC-INDUCED CATELEPSY. A.Yagi*, H. Azuma*, T. Nishimura*, T. Yamamoto*, A. Yamatodani*, and H.Wada*. (Spon: K.Kamone) Departments of Neurology and Pharmacology, Faculty of Medicine, Osaka University, Osaka 530 and Faculty of Liberal Arts, Tezukayama University, Nara 631, Japan.

The effects of histaminergic modulation on the neuroleptic induced catelepsy was studied in male ddY mice (25-30g). Catelepsy was evaluated by forcing the mouse to grasp a glass bar of 7 mm diameter set horizontally at the height of 4 cm with its forepaws, and recording the duration of the behavior and the response to tail pinching as a score of 0 to 5. Histamine was determined in brain tissue. The l.p. administrations of perphenazine (20 mg/kg) and haloperidol (10 mg/kg) induced cateleptic behavior and increases of 29% and 22%, respectively, in the brain histamine content 120 min after their administrations. Dose-dependent changes of brain histamine were observed in the dose ranges of 2 to 20 mg/kg of perphenazine and 2 to 10 mg/kg of haloperidol. Pretreatment with L-histidine (1g/kg, i.p.) enhanced the cateleptic behavior, whereas pretreatment with alpha-fluoromethylhistidine (100 mg/kg), a specific inhibitor of histamine synthesis, reduced it. Furthermore, pretreatments with mepramine (50 mg/kg, i.p.) and cimetidine (200 mg/kg, i.p.) attenuated the catelepsy. These results suggest that the histaminergic neuron system play some roles in the neuroleptic induced catelepsy.

460.7

While monkeys were performing an oculomotor delayed-response task, dopamine antagonists were injected locally into the prefrontal cortex. Monkeys fixated a central spot, and, following a delay of 1-sec, made a memory-guided saccade to the location which had been presented prior to the delay by a visual cue. Following injection of SCH23390 or haloperidol (20-80μg/1μl), the accuracy of saccades to the remembered targets decreased, and the latency of the saccade was increased. These deficits were mainly contralateral to the site, and varied with the test site. A course of 4 injections, dose dependent and also sensitive to the length of the delay period. No significant changes were observed in saccades within the same drug sessions when memory-guided saccades were impaired. In addition, injections of salbuterol (up to 100ng/10μl) failed to induce any significant performance deficits. Even a combination of memory-guided or visually-guided saccades. These results suggest that the activation of dopamine receptors, probably of D1 receptors, may play a role in the control of eye movements guided by memory of visuospatial representations in the prefrontal cortex. Supported by MH48666.

460.8

An acute injection of quinpirole (0.5-2 mg/kg) has a biphasic effect on activity, the first minutes after injection, it reduces activity, in the second hour after injection, it increases activity. In monkeys, this effect was dose dependent, and also sensitive to the length of the delay period. No significant changes were observed in saccades within the same drug sessions when memory-guided saccades were impaired. In addition, injections of salbuterol (up to 100ng/10μl) failed to induce any significant performance deficits. Even a combination of memory-guided or visually-guided saccades. These results suggest that the activation of dopamine receptors, probably of D1 receptors, may play a role in the control of eye movements guided by memory of visuospatial representations in the prefrontal cortex. Supported by MH48666.

460.4

There is a good deal of experimental evidence implicating nucleus accumbens-subpallial projections in locomotor activity. It has not been established, however, whether locomotion is mediated by subpallidal outputs to the pedunculopontine nucleus. The site of medullary subpallial locomotor region, or to the mediodorsal thalamus (Mogenson & Watanabe, 20:43-56, 1988). In the present study the role of subpallial-pedunculopontine projections in locomotor activity was investigated further. Locomotion measured in an open-field was elicited by injecting dopamine unilaterally into the accumbens or by injecting prococin unilaterally into subpallial region of rats. The effects of unilateral electrolytic lesions or of unilateral injections of kainic acid or ibotenic acid into the pedunculopontine nucleus (PPN) were investigated.

A significant and consistent reduction of locomotor activity was obtained with unilateral electrolytic or kainic acid lesions of the pedunculopontine. Electrolytic lesion of PPN had a smaller or no effect. These results provide an additional evidence that the subpallial-pedunculopontine output plays an important role in locomotor activity.
640.10


Previous studies have reported that low doses of the dopaminergic agonist apomorphine increase the frequency of play fighting in juvenile rats (30–60 days old), whereas low doses of the antagonist haloperidol decrease the frequency (e.g., Beatty W.W. et al., Pharmacol. Biochem. Behav. 20: 747–753, 1980). These results have occurred via changes in playful attack, playful defense, or both. In the present study, attack (i.e., approach to the nape of the prey), withdrawal (i.e., withdrawal of the nape) were measured separately. After 24-h isolation, 15-min after injection, ejection rats were reunited, and their behavior was videotaped under red light for 10 min. Apomorphine (0.05 and 0.3 mg/kg) significantly increased playful attack whereas haloperidol (0.15 and 0.3 mg/kg) significantly decreased attack components. Neither drug affected playful defense at these doses. Therefore, dopaminergic systems affect play fighting via the approach components, but not those of withdrawal.

640.11

THE EFFECTS OF DOPAMINE AGONISTS ON THE DRL 72-S SCHEDULE. R. Dunn, D. Jolly* and L. Seiden. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

Antidepressants increase the reinforcement rate and decrease the response rate of rats performing on a differential-reinforcement-of-low-rate 72-second (DRL 72-S) operant schedule (Seiden et al., Psychopharmacol. 86:55, 1985). The present work examines the effects of dopaminergic agonists on this antidepressant screen. PPHT, a selective D-2 agonist, decreased the reinforcement rate and increased the response rate of rats performing on a DRL 72-S schedule. Low doses of amphetamine increased the reinforcement rate and decreased the response rate of rats on a DRL 72-S schedule. Thus, dopamine autoreceptor stimulation induced antidepressant-like effects on the DRL 72-S schedule. This research was supported by NIMH MH-11191, NS-40562 (L. Seiden).

640.12


Since microinjections of amphetamine into the middle ventrolateral (MVL) portion of the striatum produce compulsive oral stereotypy (A. Kelley et al., Psychopharmacol. 95:356, 1986), we have investigated the effects of microinjections of DA agonists into this striatal area of rats with supersensitive DA receptors rendered by unilateral denervation of the nigrostriatal DA neurons with 6-OHDA. The microinjections of the mixed D1/D2 agonist apomorphine, the D1 agonist SKF 38393, or the D2 agonist quinpirole, dose-dependently induce SBB for various periods of time. The SBB induced by apomorphine is completely prevented by the systemic administration of the D2 antagonist raclopride (0.3 mg/kg) in combination with the D1 antagonist SCH-23390 (0.3 mg/kg), and that induced by SKF 38393 is partially prevented by SCH-23390 (0.3 mg/kg) alone, while that induced by quinpirole is prevented by raclopride (0.3 mg/kg) alone. These results indicate that both D1 and D2 DA receptors are involved in mediating SBB and that the MVL area of the striatum might be one of the centers involved in this abnormal behavior. Supported by NIMH 02717 and NINCIOS 09901.

640.13

THE EFFECTS OF AMPHETAMINE ON ACTIVITY AND EXPLORATION OF RATS WITH FORNIX TRANSECTIONS. S. Wood* and B. Osborne. Department of Psychology, Middlebury College, Middlebury, VT 05753.

Previously we have demonstrated that selective dopaminergic agonists reduce the activity levels of rats with fornix transactions back to control levels. The present work examines the possibility that the agonists are increasing dopamine by acting selectively on autoreceptors. Continuous lesioned rats were divided into 3 groups and received a low dose of amphetamine, a high dose of amphetamine, or a vehicle injection. Ten minutes after the injection, the rats were placed in an open field and monitored for 2 hrs. Previous findings were replicated including an increase in total activity and number of object interactions with decreased durations for fornix lesioned animals. Amphetamine-induced black freezing decreased for the high dose group as well as increased object durations with fewer object interaction bouts. The control animals displayed more stereotyped behavior than the experimental animals. Interpretations of the data are difficult because of the strong effect of the open field on control animals. Either the dose levels of amphetamine were too high or previous reports are not due to stimulating autoreceptors.
460.15
ASYMETRICAL DOSE-RESPONSE FOLLOWING MICROINJECTIONS OF APOMORPHINE IN THE NUCLEUS ACCUMBENS. M.Honig*, I.Belcheva* S.E. Starkestein, T.R.Noren, R.G.Robinson
In previous studies, we reported that rats which do not left hemisphere electrolytic lesions of the nucleus accumbens (NA) produced a sustained (30 + day) period of spontaneous running wheel hyperactivity (Kubos, et al. Brain Res. 401:147-151,1987) and that rats which do not left fronotolateral cortical suction lesions produced an increased turnover of dopaminergic NA [Starkestein, et al., Brain Res. 473:74-80,1988]. In the present experiment, male Sprague-Dawley rats (9-9) were implanted with chronic bilateral cannulae in the NA. Half the animals received injections of .32, 1, 3.2, or 10 ug of apomorphine or saline in 1 ul volume every 3 days. Post-injection activity was monitored in 2 hour test periods over a 2 hours using computerized photocell (Optimitch) chambers. A 3-way ANOVA revealed significant effects of hemisphere of injection (F(2, 480) = 12.1, p<.001), dose (F(2, 480) = 12.7, p<.001), and time (F(8, 480) = 36.1, p<.001). The only interaction that was significant was side by dose (F(4, 480) = 5.2, p<.001). Post-hoc planned comparison t-tests demonstrated significant right versus left hemisphere differences over the 2 hour test period for the .32 ug and 1.0 ug doses (t-or 8.8, p<.001 respectively). These data are consistent with our previous suggestion that subcortical asymmetries in the NA or at another post-synaptic site may play an important role in spontaneous activity and the asymmetrical response to cortical or NA lesions.

460.16
DOPAMINERGIC EFFECTS ON MATERNAL BEHAVIOR AND MILK-EJECTION IN LACTATING RATS. J.M. Stem and L.A. Taylor
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Dopamine (DA) is important in the maintenance of reproductive, stereotyped motor sequences. Moderate doses of haloperidol (HAL), a non-selective dopamine antagonist, disrupt the motivation for active components of female reproductive behavior (hops, dams; i.e., proceptivity, while leaving the lordosis reflex (i.e., receptivity) intact (Hansen, Stanfield, & Everitt, Neuroscience, 1981). Accordingly, we tested whether a similar disruption of female reproductive behavior occurs in the mother. First, we investigated the effects of HAL on maternal behavior (cervix, licking and nursing behavior) observed in response to the opiate antagonist naltrexone (20 mg/kg, i.p.).

Next, we examined the effect of HAL on the milk-ejection reflex. Since the milk-ejection reflex is involved in the feeding of pups, we tested the effect of HAL on milk-ejection. The milk-ejection reflex is induced by the suckling stimulus (i.e., pups). Thus, two mechanisms may provide milk-ejection: a suckling reflex (endogenous suckling) and endogenous prolactin release. In these studies, HAL, given during the suckling phase, was observed to decrease milk-ejection.

Finally, we investigated the effect of HAL on the milk-ejection reflex during the suckling phase. The results of these studies indicate that HAL may have a role in the regulation of suckling behavior.

460.17
DI AND D2 RECEPTORS IN THE MPOA REGULATE COPULATION AND PENILE REFLEXES IN MALE RATS. E. Hull, R. Eaton*, T. Thompson*, T. Burnett* and V. Markowski*
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We have reported that the D2 agonist LY134952 (quinolone) or the D1 antagonist SCH-23390, microinjected into the medial preoptic area (MPOA) of male rats, delayed the onset of copulation and slowed its rate, but then lowered the ejaculatory threshold (decreased the number of intromissions required to trigger ejaculation), such that the number of erections and penile movements in reflex tests of restrained, supine animals. Seminal emissions were increased. Thus, the delayed onset and slowing of copulation may have reflected an impairment in erectile function, which is primarily controlled by the parasympathetic system. The decreased ejaculatory threshold in copula may have been associated with an increase in sympathetically mediated seminal emission.

The D2 antagonist raclopride in the MPOA also delayed the onset of copulation and decreased its rate, and lowered ejaculatory threshold, similar to the effects of the D2 agonist. Preliminary data suggest that its effects on penile reflexes are also similar to those of the D2 agonist. Thus, it appears that any shift in the D1/D2 ratio in the MPOA may alter the balance of autonomic influence on reflex penile mechanisms, and thereby alter copulatory parameters.

Supported by NIH grant #0826.

460.18
DOPAMINE-2 AGONIST, LY134952, ACTS CENTRALLY TO STIMULATE MALE SEX BEHAVIOR OF RHEUSUS MONKEYS. S. M. Pomerantz.
Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Recent studies demonstrated that dopamine agonists facilitate male sexual behavior of rhesus monkeys (Soc. Neurosci. Abstr. 14: 664, 808, 1988). In order to determine whether the D2 agonist, LY134952, acts centrally or peripherally to stimulate male sexual behaviors, we examined the effect of dopamine antagonists, domperidone (DOM, peripheral action only) or haloperidol (HAL, central and peripheral action) to block the sexual response to LY134952. Male monkeys were pretreated with 100 µg/kg HAL, the centrally active dopaminergic antagonist, which markedly reduced the sexual behavior of monkeys (penile erection and masturbation) were assessed in a testing situation in which monkeys were presented with a female monkey they could see, hear and smell, but not physically contact. Eight monkeys were rotated through treatments in which they received either 50, 100 or 200 µg/kg DOM or vehicle forty min prior to testing and 5 µg/kg LY134952 or vehicle 10 min prior to testing. LY134952 facilitated penile erection (p<0.001) and masturbation (p<0.05) over vehicle levels, both in monkeys pretreated with DOM and those not pretreated with DOM. Evidence that DOM was acting as a peripheral dopamine antagonist was obtained from two male monkeys in which 100 µg/kg DOM markedly inhibited prolactin release. Administration of LY134952 following DOM in these monkeys failed to reduce prolactin to control baseline levels. In contrast to DOM, the centrally active dopamine antagonist, HAL, blocked the sexual response to LY134952. In monkeys pretreated with 10 µg/kg HAL 50 min prior to LY134952, male sexual behavior was markedly reduced compared to monkeys pretreated with vehicle prior to LY134952. These results indicate that systemically administered dopamine agonists act centrally to stimulate male sexual behavior of rhesus monkeys.
The depression of synaptic transmission by the glutamate analog L-2-amino-4-phosphonobutyrate (L-APB) in certain hippocampal pathways is thought to occur presynaptically. Whole-cell recordings were obtained from embryonal rat hippocampal CA1 and entorhinal cortical neurons maintained in culture for 4-14 days. Sodium currents were blocked by TTX (0.5 µM) and outward currents by internal cesium (150 mM). ATP (2 mM), to reduce rundown of calcium currents, and GTP (1 mM) were included in the internal solution. In the presence of calcium (2 mM) or barium (2 mM) depolarizing steps from -70 to 40 mV produced inward currents that were completely blocked by external cadmium chloride (200 µM). L-APB (10-100 µM), applied by fast-perfusion, rapidly and reversibly reduced the peak calcium current by 5-25% in 16/25 L-APB-exposed neurons. L-glutamate (1 µM) mimicked the action of L-APB under conditions in which activation of NMDA, kainate and quisqualate receptors was prevented by kynurenic acid (200 µM), D-2-amino-5-phosphonovalerate (20 µM) and 6-cyano-7-nitroquinazoline-2,3-dione (20 µM). A reduction in calcium entry into presynaptic neurons may explain the depression of synaptic transmission induced by L-APB.

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461.3 KAINIC ACID-INDUCED SEIZURES: EFFECTS OF ANTICONVULSANTS AND NIFEDIPINE. D.E. Braun* and W.J. Freed. Department of Neurosurgery, Naval Hospital Bethesda, MD 20814* and NIMH Research Group, University of Calgary, Calgary, Alberta T2N 4N1.

The kainate and kainic acid excitatory amino acid receptors are related, and antagonists of the kainic acid and quisqualate receptors have recently been identified (Science 241:701, 1988). Depakene and glutamic acid (GAU) mimicked the action of kainate (KA). Kainate and quisqualate (QA) receptors were prevented by kynurenic acid (200 µM), D-2-amino-5-phosphonovalerate (20 µM) and 6-cyano-7-nitroquinazoline-2,3-dione (20 µM). In all cells, the current activated by pressure injection of KA (100µM-10mM) was significantly reduced by bath application of 100µM QA, but not by GAU or AMPA. Identical results were observed when the methods of application were reversed. The fractional reduction in KA-activated current by 100µM QA was approximately constant for 10µM-10mM KA, suggesting that the interaction between QA and KA was not competitive.

We have begun to characterize this functional kainate receptor in order to determine the structural requirements for agonist-induced seizures. A dosage of 50 mg/kg produced about a 50% decrease, primarily as a result of decreases in the length of individual seizure episodes. Thus there are substantial differences in the agonists of kainate and kainic acid-induced seizures. The ineffectiveness of clinical anticonvulsants in this model could be related to the unresponsiveness of certain intractable seizure patients.

Current Gated by Kainate in Rat Dorsal Root Ganglion Neurons: Con A abolishes desensitization. J. E. Huettner, Dept. of Neurobiology, Harvard Med. School, 25 Shattuck St., Boston, MA 02115. Primary afferent C fibers in rat dorsal roots are depolarized by the excitatory amino acid, kainate (Agrawal and Evans 1986 Br. J. Pharm. 87:345). Under whole-cell voltage clamp, Kain and domoate increase the conductance of a subpopulation of small diameter (<35 µm) sensory neurons, freshly dissected from rat DRG's. The I-V is linear from -100 to +60 mV and reverses near 0 mV with internal Ca2+ and K+ and internal Cl- (May and Takahashi 1988 Pfluegers Arch). Kynurenic acid (200 µM) blocked responses to Kain and Dom with rapid onset and recovery. Glu, Quis, Asp and NMDA do not evoke current, but simultaneous application of Glu or Quis antagonizes responses to Kain or Dom. Expression of Glu or Quis also suppresses responses to a subsequent application of Kain or Dom (1/2 recovery=2-3 min). Incubation with 10 µM Con A abolishes desensitization to Kain and Dom, both Glu and QA responses to Kain and Dom conductance and no longer suppress responses to Kain or Dom.

supported by NIH grants NS21419 and the McKnight Foundation.

Hydrogen ion inhibits the quisqualate/kainate response by protonation of histidine groups in isolated catfish horizontal cells. B.R. Chiitzen and E. Hida*. Dept. Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

The whole cell patch technique was used to measure kainate (KA) or quisqualate (QA) induced membrane current from enzymatically dissociated catfish cone horizontal cells. Increasing the extracellular hydrogen ion concentration reversibly decreased the agonist induced membrane current. The pK was estimated from a Titration curve in which the membrane current was measured as a function of pH in a constant concentration of agonist.

The specific sulphydrol reagent iodoacetic acid (10 µM) had no effect on the agonist induced membrane current. These results suggest that the imidazole ring of the histidine amino acid is associated with the ion permeation process of the QA/KA channel protein.

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Pharmacological characterization of a kainate (KA) receptor expressed in Xenopus laevis oocytes injected with rat brain mRNA. M.A. Balabanoff Jr., G. B. Watson, M.P. Boganoff, C.J. Doppke* and T.T. Ludden. CNS Disease Research, G. D. Searle & Company, and #Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Xenopus laevis oocytes express a wide variety of biological activities encoded by exogenous mRNA, including numerous receptors and ion channels. Kainate elicits an inward current in oocytes injected with rat brain mRNA. The kainate analog domoate (DOM) was more potent (EC50=10 µM) than KA. The N-methyl-D-aspartate (NMDA) receptor antagonist 6,7-dinitro-quinoxaline (DNQX; IC50=0.3-0.4 µM), and kynurenate (KYNA; IC50=90 µM). The N-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-7-phosphonoheptanoate (D-AP7) did not block responses to KA. The kainate analog doxapram (DOM) was more potent (EC50=10 µM) than KA. DOM responses were blocked by QA and DNOX at concentrations similar to those which blocked KA responses. In addition, we have tested the activities of other KA analogs, including 5-kainate and dihydrokainate, as well as several non-NMDA agonists (e.g. willardiine and 5-bromo-willardiine). These characteristics demonstrate the presence of a low affinity KA receptor in oocytes injected with rat brain mRNA.
461.7 QUISQUALIC ACID MEDIATED INTRACELLULAR CALCIUM MOBILIZATION IS INHIBITED BY PHORBOL ESTERS BUT NOT BY PERTUSSIS TOXIN. Shawn N. Murphy*, James A. Holton†, and Richard J. Miller, (Spon: W.J. Niemer) Un. of Chicago, Chicago, IL 60637.

We have previously demonstrated that fura-2 based microspectrofluorimetry that mouse hippocampal neurons in monolayer culture posses a glutamate receptor whose activation leads to mobilization of Ca2+ from intracellular stores and does not appear to be linked to a cation channel. In addition to glutamate, quisqualic acid (QA) and ibotenate activated this receptor but not a-amin-o-3-hydroxy-5-methylisoxazole propionate (AMPA). Furthermore, the stimulatory effects of glutamate and QA were not blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). We have now investigated the nature of the transduction mechanism involved in this process. Treatment of the neurons with 2.5 µg pertussis toxin (PTX) for 48 hours had no effect upon QA stimulated Ca2+ mobilization. Carbachol (CCh) and phenylephrine stimulated Ca2+ mobilization were also unaffected by PTX pretreatment although 2-chloro-adenosine inhibition of Ca2+ currents was mobilized by > 90%. This appears that the pathway mediating QA induced Ca2+ mobilization may be subject to an inhibitory protein-kinase C feedback as are many other systems involved in inositol-1,4,5-trisphosphate production.

461.8 EFFECTS OF QUISQUALIC AND NMDA ON PHOSPHODIYLYSINOSITOL TURNOVER IN RAT CEREBELLUM. Josiane Fichot-Cheney and Sheryl S, Smith, Dept. of Anatomy, Helmholtz Univ. Muenchen, Muenchen, Germany. (Spon: J.Kinnier) Un. of Chicago, Chicago, IL 60637.

We have previously shown that the excitatory amino acid (eaa) Quisqualic (Q) and NMDA produce long-term synergistic effects on Q-evoked excitatory responses of cerebellar Purkinje cells recorded in vivo. In the present study, we have utilized a microspectrofluorimetric technique to investigate the eaa induced mobilization of PI in the cerebellum. PI hydrolysis was measured in 160 x 65 µm chunks of cerebellar tissue from 6-9 day old female Long-Evans rats. Uptake of [3H]myo-inositol took place in the presence of 1 mM EDTA, and the subsequent release of PI in FI was measured with a sensitized filter. PI hydrolysis was then measured and the release of PI was then measured by the excitation scan with apparent emission of 460 nm. Phosholipase A2 alone increased basal PI turnover by 17% and had no effect on Q-stimulated values. However, administration of bicarinate almost completely abolished the stimulus effects on this parameter. In parallel, the ability of Q to increase PI turnover during stimulation by 70% in the presence of 100 µM Q was almost completely abolished. This indicates that under conditions of reduced GABAergic tone, the eaa Q and NMDA produce synergistic effects on the turnover of PI, a second messenger system which may lead to long-term changes in neuronal function in the developing cerebellum. Supported by NS30869 to SS.

461.9 DIFFERENTIAL EFFECTS OF EXCITATORY AMINO ACIDS ON CYCLIC AMP ACCUMULATION IN RAT BRAIN SLICES. A. Filc, W. Kania†, J. Perkowska, J. Vejulmans*, S. Jakoła, Inns. Institute of Pharmacology and Toxicology, Polish Academy of Sciences, 31-343 Krakow, Poland; Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Poland; Nova Pharmaceutical Corp., Baltimore, MD 21224-2788.

The effects of excitatory amino acids (eaa) on cAMP accumulation in rat brain cortical slices were measured using a prelabeling technique. Glutamate (0.1-5.5 mM), which by itself caused a slight increase in basal cAMP accumulation, inhibited up to 60% the cAMP response to NE (200 µM), in a concentration-dependent manner, suggesting that it modifies the alpha component of the NE response. The inhibitory effect of glutamate was mimicked by quisqualic (0.01-10.0 mM), but not by NMDA or kainate, at concentrations up to 5 mM. The inhibitory effect of glutamate was insensitive to CFF (10 µM), supporting the notion that the glutamate effect is not mediated by NMDA receptors. In contrast to glutamate, ibotenate (0.01-1.0 mM), which alone caused a modest increase in basal cAMP levels, enhanced the cAMP response to NE more than 5-fold. These findings provide an additional mechanism by which eAA can influence second messenger production in brain, and the data suggest that a quisqualic receptor mediates the inhibitory effect of glutamate on NE-stimulated cAMP accumulation.

461.10 KAINATE EVOKES THE RELEASE OF ENDOGENOUS GLYCINE FROM STRIATAL NEURONS IN PRIMARY CULTURE. S. Weiss and L. Bauce, Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1.

The actions of selective excitatory amino acid (eaa) agonists and other depolarizing agents on the release of endogenous glycine and GABA from striatal neurons in primary culture was examined. The concentrations of endogenous amino acids released into the extracellular medium was determined by pre-column derivatization and separation with high performance liquid chromatography. Baseline levels of glycine and GABA, recorded from the neurons into the extracellular medium during a 3 min period, were 0.73±0.07µM and 0.09±0.02µM, respectively. When striatal neurons were exposed to 50mM KCl for a 3 min period, glycine and GABA levels increased to 1.11±0.19µM (1.5-fold) and 1.96±0.11µM (2.8-fold), respectively; 50% of these increases were dependent upon the presence of extracellular calcium. Exposure of striatal neurons to 100µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) produced 20% increase in PI turnover above basal values in the presence of 100µM KCl, indicative of a Q-evoked effect similar to that observed after multiple applications of this amino acid. In the non-blocked condition, baseline application of Q evoked a 20% increase in PI turnover above basal values, a level similar to that obtained with a single dose of Q. In contrast, 17µM (0.1µM) decreased Q-evoked PI turnover by 20%. Other reports have indicated that GABA blockers permit the induction of LTP in the cortex. These findings are consistent with the notion that under conditions of reduced GABAergic tone, the eaa Q and NMDA produce synergistic effects on the turnover of PI, a second messenger system which may lead to long-term changes in neuronal function in the developing cerebellum. Supported by the Medical Research Council of Canada.

461.11 MUTAGNE AND NMDA ON PHOSPHODIYLYSINOSITOL TURNOVER IN RAT BRAIN SLICES. A. Filc, W. Kania†, J. Perkowska, J. Vejulmans*, S. Jakoła, Inns. Institute of Pharmacology and Toxicology, Polish Academy of Sciences, 31-343 Krakow, Poland; Nova Pharmaceutical Corp., Baltimore, MD 21224-2788.

The actions of excitatory amino acids (eaa) on cAMP accumulation in rat brain cortical slices were measured using a prelabeling technique. Glutamate (0.1-5.5 mM), which by itself caused a slight increase in basal cAMP accumulation, inhibited up to 60% the cAMP response to NE (200 µM), in a concentration-dependent manner, suggesting that it modifies the alpha component of the NE response. The inhibitory effect of glutamate was mimicked by quisqualic (0.01-10.0 mM), but not by NMDA or kainate, at concentrations up to 5 mM. The inhibitory effect of glutamate was insensitive to CFF (10 µM), supporting the notion that the glutamate effect is not mediated by NMDA receptors. In contrast to glutamate, ibotenate (0.01-1.0 mM), which alone caused a modest increase in basal cAMP levels, enhanced the cAMP response to NE more than 5-fold. These findings provide an additional mechanism by which eAA can influence second messenger production in brain, and the data suggest that a quisqualic receptor mediates the inhibitory effect of glutamate on NE-stimulated cAMP accumulation.


Excitatory amino acids (eaa) participate as transmitters in the retina. We have previously demonstrated the presence of pre-synaptic eaa receptors which mediate the depolarization-evoked release of eaa. We have also demonstrated natural changes in eaa synaptic receptors during ontogeny in the chick retina, which could be related to the difference in the distribution of eaa receptors during development. Since the activity of these receptors depends on the availability of transmitter, which in turn is under pre-synaptic control, we have investigated the role of eaa and other natural modulators of eaa nerve terminals, e.g., dopamine, in the eaa-mediated release of eaa. We have developed a microspectrofluorimetric technique to measure the eaa-evoked release of transmitter from chick embryo retina. The results of our experiments suggest that the eaa-evoked release of transmitter from chick embryo retina is under the control of dopaminergic and GABAergic systems. The eaa-evoked release of transmitter is reduced by 30-40% in retina that was pretreated with a partial agonist of dopamine, while 60-70% reduction is observed in retina that was pretreated with an antagonist of GABAergic systems. The eaa-mediated release of transmitter is also reduced by 30-40% in retina that was pretreated with a partial agonist of dopamine, while 60-70% reduction is observed in retina that was pretreated with an antagonist of GABAergic systems.
MODULATION OF THE RELEASE OF ENDOGENOUS GLUTAMATE FROM RAT BRAIN SYNAPTOPLASM BY PUTATIVE PRESYNAPTIC RECEPTORS
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Rat brain synaptosomes were used as a model system for characterizing the properties of putative pre-synaptic receptors. Glutamate, a classical excitatory amino acid neurotransmitter, has been shown to elicit a Ca^2+mediated exocytosis of glutamate from synaptosomes. In these experiments, synaptosomes were treated with various excitatory amino acids as well as VIP, forskolin, and/or 8-bromo-cAMP, and then the extracellular levels of glutamate released were measured using a radioimmunoassay technique. The results of these experiments indicate that glutamatergic stimulation of synaptosomes is not a simple monophasic process but is characterized by a biphasic release, with a peak release at 2 min followed by a second lower peak release at 30 min. The first peak release is relatively insensitive to TTX, Ca^2+ removal, and blockade of voltage-sensitive Ca^2+ channels, whereas the second peak release is blocked by TTX and Ca^2+ removal, and is sensitive to blockade of voltage-sensitive Ca^2+ channels. These findings suggest that there are two different mechanisms for the release of glutamate from synaptosomes, one that is Ca^2+-independent and one that is Ca^2+-dependent. The Ca^2+-dependent mechanism is characterized by a biphasic release, with a rapid peak release followed by a slower, more sustained release. The Ca^2+-independent mechanism is characterized by a single, rapid release. These findings have important implications for our understanding of the mechanisms of neurotransmission and the regulation of synaptic transmission.

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Intracellular Ca2+ changes were studied in astrocytes cultured from the CA1 region of the rat hippocampus using time-lapse video microscopy, the Ca2+ indicator Fura-2 and FITC optics. Intracellular Ca2+ responses were evoked upon bath application of 1-1000 nM glutamate in all astrocytes. After this initial rise three responses were seen. In one response, the cell Ca2+ oscillates with nearly constant amplitude and frequency (period ~ 15 s, 3.3 ± 0.5 Hz) during the experiment (5-10 min). In another type, the cell begins to oscillate with constant frequency while the amplitude dampens to an elevated baseline. In the third type, cells do not oscillate. Calcium glia may also participate in expansive Ca2+ waves that propagate from cell to cell and radiate from initiation centers. In Ca2+-free/3 mM EGTA Ringer's oscillations are seen, though damped, suggesting they are partly due to cellular Ca2+ release. Quisqualic acid (100 μM) induces transient oscillations and similarities to 100 μM glutamate while NMDA (100 μM) with glycine (10 μM) has no effect. Kainate (100 μM) causes a step-like rise in Ca2+. Neither the selective NMDA antagonist APV (1 mM) nor the putative glutamate receptor antagonist APB (1 mM) could antagonize glutamate-induced effects. However, the selective non-NMDA antagonist, CNQX (10-100 μM), markedly attenuates the oscillations and waves. Blocking the sodium-dependent glutamate uptake system by choline substitution enhances the amplitude of glutamate-induced oscillations. The above responses cease quickly when the agonist is removed. These results suggest astrocytes possess receptors preferring kainate and quisqualate. The oscillations are consistent with models of quisqualate-activated phosphoinositide turnover. These findings indicate that intercellular communication, possibly through gap junctions, can establish long-range Ca2+ signalling in glial networks.

LOCALIZATION OF THE CEREBELLAR KAINATE RECEPTORS ON BERGMANN GLIAL CELLS. N. Eshhar**, J. D. Roberts***, V. L. Ichterwegh† and P. Somogyi‡.


A high density of kainate receptors has been reported to be present in the cerebellar molecular layer of the goldfish (Eshhar et al. Brain Res., 476, 77-79, 1989) and the chick (Berg et al. EMBO J. 7, 1769-1776, 1988). The precise cellular and subcellular distribution of these kainate receptors has now been studied by EM immunocytochemistry using the monoclonal antibody 1K-50 directed against the purified kainate receptor protein. Both postsynaptic and presynaptic immunoreactive and immunogold methods were used. Immunoreactivity was found associated only with Bergmann glial cells. Intracellularly, immunoreactivity is observed in the endoplasmic reticulum, Golgi apparatus and lysosomes, representing putative sites of the synthesis, glycosylation and degradation of the kainate receptor. Extracellular immunoreactivity appears to be uniformly distributed along the Bergmann glial plasma membrane as confirmed by high resolution light microscopy. Bergmann glial processes form nets to the parallel fibre/purkinje cell spine synaptic complexes. The presence of kainate receptors on Bergmann glia in close association with the spine synapses provides a basis for parallel fiber signalling to the Bergmann glia, presumably via glutamate.

SODIUM-DEPENDENT D-ASPARTATE BINDING IS NOT A MEASURE OF PRESYNAPTIC NEURONAL UPTAKE SITES IN AN AUTORADIOGRAPHIC ASSAY. J. H. Greenaway, D. B. Higgins* and A. B. Young. University of Michigan, Ann Arbor, MI 48109.

Binding of D-[3H]aspartate to rat brain was examined in an autoradiographic assay. Binding was dependent on the presence of sodium ions, but not chloride ions, and was optimal at 2°C. D-Aspartate binding reached equilibrium in 20 min and remained stable for 45 min. Displacement was rapid with a t1/2 of 20 sec, but was not as fast as anticipated, perhaps because of some sequestration of ligand. Binding in striatum had a KB of 6.8 ± 1.2 μM and was 49.9 ± 8.6 pmol/mg protein. L-Glutamate, unlabelled D-aspartate, and D,L-threo-hydroxyaspartic acid, each competed for D-[3H]aspartate binding with IC50s of 7.02 ± 4.3 μM, 5.6 ± 1.9 μM, and 2.54 ± 1.03 μM, respectively. N-methyl-D-aspartate, quisqualate, and kainate had no affinity for this site. The regional distribution of binding did not conform to that of neuronal uptake sites described by others. Striatal D-aspartate binding was unaffected by unilateral decortications or striatal quinolinic acid lesions. NMDA, quisqualate, and kainate receptors were reduced by 80-90% by quinolinic lesions. The lesion studies strongly suggest that this site is not associated with either presynaptic glutamatergic afferents or intrinsic neurons of the striatum; it may be associated with glia.

CHARACTERIZATION AND DISTRIBUTION OF 3H-MK-801 BINDING SITES IN THE RAT BRAIN. S. Subramaniam and P. McGonigle, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Kinetics and optimal conditions for the binding of 3H-MK-801, a non-competitive antagonist at the NMDA receptor, were determined using sections of brain prepared from rat brain. 30 μM thick sections were incubated at 22°C with 3H-MK-801 (2-50 nM) in 30 mM EPSS containing 100 μM glutamate and 100 μM glycine. Non-specific binding was defined with 1000 μM glutamate and 100 μM glycine. Equilibrium was reached at 150 min. Sections were then rinsed in ice-cold buffer, wiped off and counted in scintillation fluid. Equilibrium binding to hippocampus CA1 region of the hippocampus was at 0.7% of that to hippocampus CA1 region of the hippocampus at 100 μM. 3H-MK-801 binding sites was determined in 34 regions at a concentration of 32 nM. These values corresponded to the density of 0.97 ± 0.42 pmol/mg protein in the ventral hippocampus and 0.31 pmol/mg protein in the dorsal hippocampus. The density of 3H-MK-801 binding sites was determined in 34 regions at a concentration of 32 nM. These values corresponded to the density of 0.97 ± 0.42 pmol/mg protein in the ventral hippocampus and 0.31 pmol/mg protein in the dorsal hippocampus.
462.3
A UNIQUE GLUTAMATE BINDING SITE IN AN AUTORADIOGRAPHIC ASSAY. D.S. Higginbotham, J.J. Crepax, M.C. Giudicelli, P.M. Penney, Dept of Pharmacology, University of Michigan, Ann Arbor, MI 48104.

Glutamate receptor pharmacology identifies 3 postsynaptic receptor types: N-methyl-D-aspartate, quisqualate and kainate, each of which is labelled by [3H]glutamate. We describe a unique neuronal glutamate recognition site measured in the presence of saturating concentrations of NMDA, quisqualate and kainate in rat brain. In Tris buffer binding was enhanced by the presence of chloride and calcium ions and was maximal at 2°C. Glutamate bound rapidly, reaching equilibrium by 15 min and dissociated with a t1/2 of 24 sec. Kinetic analysis yielded a Kd of 0.13±0.04nM while scatchard analysis gave a KD of 1.05±0.20nM and a Bmax of 2.22±0.36pmol/mgprot, with a Hill coefficient of 0.9±0.04. Binding was highest in outer cortical lamina, dentate gyrus and striatum. A wide variety of glutamate agonists and antagonists, as well as SITS and DIDS, did not alter binding. Binding in striatum was unaffected by ipilharal dissection but was reduced by 65% in striatal granule neurons. These results suggest that this is a unique postsynaptic neuronal site. Its function is not yet known. Supported by USPHS grants NS19613, AG06155 and NS07222.

462.5

We have investigated the pharmacological properties and regional distribution of binding of the glutamate receptor agonist [3H]RS-3-amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([3H]AMPA) in rat brain using quantitative autoradiography.

[3H]AMPA binding was highest in the hippocampus, cortex and cerebellum. Quisqualate and the quinoxalinedione compounds CNQX and DNQX were the most potent displacers of AMPA binding with IC50 values in the nanomolar range. Glutamate and BOAA also displaced AMPA binding with IC50 values in the low micromolar range. Nicotinic and muscarinic agonists had no effect on AMPA binding. Regional analysis confirmed that AMPA binds to a subpopulation of quisqualate-sensitive glutamate binding sites.

Potassium chloride (KCl) increased [3H]AMPA binding at all concentrations of KCl tested (0-100mM). In the presence of 100mM KCl, Scatchard analysis revealed a non-single binding site. However, in saturation experiments using very high concentrations of [3H]AMPA, a second binding site was detected in the absence of KCl. The regional distributions of [3H]AMPA binding in the absence of KCl and in the presence of 100mM KCl were identical (r = 0.6). Scatchard analysis performed in the presence of intermediate concentrations of KCl (1 and 10mM) suggest that KCl stimulated [3H]AMPA binding by increasing the affinity of a low-affinity site. These data suggest that AMPA binding sites can exist in two conformations, and that the equilibrium between these two sites can be influenced by KCl. Supported by USPHS grant NS 19613 and NIH NSRA 5T32.

462.6
EFFECTS OF AGE ON GLUTAMATE DISPLACEABLE KAINIC ACID BINDING IN MOUSE BRAIN. J.C. Matthews, Department of Pharmacology and Research Institute of Pharmaceutical Sciences, School of Pharmacy, Univ. of Mississippi, University, MS 38677.

Female mice at 2.5, 10 and 20 months of age, produced at the University of Michigan by brother-sister mating, were purchased from Charles River Breeding Laboratories, Wilmington, MA, 19705. Studies of the kindling model suggest that EAA-mediated neurotransmission may contribute to long-term potentiation in animal models. Therefore, we have used conventional radioligand binding and receptor autoradiography to measure the binding of ligands to NMDA receptors in mouse brain. The present study was designed to determine whether there is a significant change in the density of NMDA receptors with age. Male mice at 2.5, 10 and 20 months of age were used. The results suggest that quisqualate-sensitive [3H]-glutamate binding sites were significantly decreased (33% and 61%, respectively, in areas G3A and G1A) in dentate gyrrus stratum moleculare from these same specimens, but not significantly different in G3A or G1A. The decreased [3H]-TCP and NMDA-displaceable [3H]-glutamate binding may reflect a down-regulation of NMDA receptor/channel function in surviving neurons. The increased [3H]-NBQX binding in epileptic human hippocampi may indicate enhanced function of the same type of synapses that underlie long-term potentiation in animal models.

462.7

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462.4

[3R]-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) is a selective glutamate agonist for the quisqualate class of excitatory amino acid receptor. However, AMPA displaces only a portion of quisqualate-sensitive [3H]glutamate binding in rat brain. We investigated the properties of this "AMPA-sensitive, quisqualate-displaceable [3H]glutamate binding" ([3H]AMPA binding) in rat brain using a quantitative autoradiographic assay.

AIQGB was measured in 50 mM Tris HCl buffer plus 2.5 mM CaCl2 and 10 μM AMPA. Non-specific binding was determined in the presence of 2.5 μM quisqualate. AIQGB was increased by 4'-pitol, distinguishing it from other quisqualate-sensitive glutamate binding processes. Similarly, thionamide blockers (i.e. KC2 > 100 μM) were cytotoxic, kynurenamethyl, L-APB, BOAA, CNQX and DNQX. Effective displacers of AIQGB were (with approximate IC50 values) quisqualate (10-20 μM), glutamate (200-300 μM) and ibotenate (2.3α M).

AMPA-sensitive, quisqualate-sensitive [3H]glutamate binding may represent binding to the novel type of quisqualate receptor believed to be linked to phosphoinositide metabolism. Supported by USPHS grant NS 19613 and NIH NRSA 5T32.

462.8

Quisqualate and AMPA ([3R]-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) act as potent agonists at a subset of excitatory amino acid receptors linked to phosphoinositide metabolism.

The number and density of binding sites in the cerebral cortex remained constant. In the cerebral cortex the affinity constant remained constant with age. It can be concluded that the density of receptors in the forebrain decreased with age.

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Aging is associated with a reduction in several forms of neuronal plasticity; older rats learn spatial tasks and kindle at a slower rate than young adults. Pharmacological studies suggest that activation of N-methyl-D-aspartate (NMDA) receptors is critical to these events. A change intrinsic to the NMDA receptor-gated ion channel may be responsible for age-related deficits. To test this idea, we measured the binding of \(^3\)H-N-methyl-D-aspartate (NMDA) to the NMDA channel of hippocampal membranes from 3 and 24 month old Fisher 344 male rats. Equilibrium analysis of TCP binding isotherms (T=30°C, 500 min) showed a decrease in maximum binding with no change in Kd.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Bmax (pmol/mg)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.14 ± 0.37</td>
<td>19.9 ± 1.7</td>
</tr>
<tr>
<td>24</td>
<td>1.95 ± 0.17</td>
<td>16.3 ± 1.9</td>
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</tbody>
</table>

Values are mean ± S.E.M. *Significantly different by Student's t-test, p<0.02, n=4.

This reduction in the number of NMDA channels supports the idea that a change intrinsic to this receptor/channel complex contributes to the decreased plasticity observed in the aged brain.

462.10

AGE-RELATED CHANGES OF AMINO ACID RECEPTORS IN THE RAT BRAIN STUDIED BY IN VITRO AUTORADIOGRAPHY. R. Miyoshi, S. Kito, W. Douber* and S. Katayama*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

Glutamate appears to be a major excitatory transmitter in the mammalian central nervous system. Central excitatory amino acid receptors have been thought to be divided into three subclasses referred as N-methyl-D-aspartate (NMDA), quisqualic acid (QA) and kainic acid-sensitive sites. Among these subtypes, NMDA sites are proposed to be involved in various central events such as neuronal plasticity, hypoxic neuronal death and long-term potentiation. In addition, strychnine-sensitive glycine receptors seem to be strongly related to NMDA receptors.

In the present study, autoradiographic distributions of \(^3\)H-glu (a NMDA receptor antagonist), \(^3\)H-AMPA (an agonist for QA receptors) and \(^3\)H-glycine were investigated in the rat brain. Binding sites of each ligand whose Kd values were nanomolar-order were obtained by Triton X-100 treatment of tissue sections. High densities of each ligand were observed in the hippocampus, cerebral cortex, amygdala, olfactory tubercle and striatum. In addition, alterations of receptor distributions were also studied using the aged brain. Amino acid receptors in the hippocampus were decreased in parallel with aging. Hippocampal amino acid receptors may play an important role in brain aging.

462.11

ANTI-IDiotypIC ANtIBodies AS INTERNAL IMAGES OF GLUTAMATE AND THEIR IMMUNOCYTOCHEMICAL APPLICATION IN THE RAT BRAIN. G. Campstron, P. Duboucq, M. Geoffroy and A. Calas*. Neuroimmunology, IBCN-CNRS, Bordeaux, FRANCE.

Idiotype poly- and monoclonal anti-conjugated glutamate (Glu) antibodies or Ab2 allowed us to induce anti-idiotypic Glu antibodies or Ab2 in rabbits after alternative immunization. These latters were affinity purified by Protein A-Sepharose. Using Glu antibody or Ab2 allowed us to induce anti-idiotypic Glu antibodies or Ab2. Combined treatments with Ab2 and Glu-conjugates induced a sharp decrease of immunoreactivity. These data suggest that our anti-idiotypic antibodies can specifically recognize Glu receptors and open new possibilities for their ultrastructural and biochemical characterization.

462.12


In vitro autoradiography was used to monitor the response of two subclasses of excitatory amino acid receptors, N-methyl-D-aspartate (NMDA) and quisqualic acid (QA), in the rat hippocampus after unilateral lesions of the entorhinal cortex (EC). In response to EC lesions changes in NMDA and QA receptor binding levels were found not only in the outer two-thirds of the ipsilateral molecular layer (IML) but also in the inner IML indicating that the response of receptors is not restricted to directly denervated areas. An early decrease (5-20%) in the binding density of NMDA and QA receptors in the IML during the first week postlesion was followed by an increase in binding levels. Thirty to sixty days postlesion the binding levels of both receptors in the IML were higher than those in unoperated rats. The response of NMDA receptors (20-50% increase over control) was more pronounced than that of QA receptors. An increase in NMDA receptor binding levels (10-15% over control) was also found in the contralateral ML three to sixty days postlesion. No such changes were observed for QA receptors.

The data indicate that NMDA and QA receptors are differentially regulated in response to deafferentation. Comparison of these results to the time course of deafferentation and reinnervation suggests that the observed changes may be important components in the functional restoration of partially damaged circuitry.
INHIBITION OF GLUTAMATE-INDUCED FURING IN HIPPOCAMPAL SLICES BY LOW GLUCOSE OR BY AMMONIUM IONS. Fan Ping* and J.C. Sprecher. Dept. of Physiol. & Biophys., Dalhousie Univ., Halifax N.S.,B3H 4H7 Canada.

Synaptic transmission in hippocampal slices is depressed in low glucose medium (Cox & Bachevalier, Brain Res. 992,297(1982)) or by NH4+ (Theoret et al. Neurosci. Lett. 14:798,1985). Whether this depression is pre- or postsynaptic is controversial. To test for a post-synaptic block, firing of units in the pyramidal layer of CA1 area induced by 100 msec pulses of electro-physiologic glutamate was tested. Lowering glucose from 5 to 0.2 mM glutamate induced firing by about 70%, but 2 or 5 mM NH4Cl reduced it by only about 20%. If however, 25 µM bicuculline was present, which increased the response of neurons to glutamate, the effectiveness of NH4Cl more than doubled. These observations agree with findings that neither low glucose (Szeber & Ojeda, J. Neurosci. 1:265,1981) nor 5 mM NH4Cl (Butterworth et al. Reciprocal Dopaminopathy, Humana Press, in press) inhibit stimulus-induced release of excitatory amino acides from hippocampal slices and suggest that synaptic transmission is blocked at a post-synaptic site. To reveal the blockade by NH4Cl of glutamate-induced excitation, GABA-ergic inhibition has to be reduced, since, by interfering with the Cl- pump, NH4Cl also decreases this inhibition (Bassane, Brain Res. 210:931,1981). (Supported by the NRC of Canada.)


During the past several years, the NMDA antagonist radioligands [3H]CPP (3- (2-carboxy-2-cyclopropylamino-4-phosphonomethyl phosphonic acid), and [3H]CGS 19755 (3- (4-phosphonomethylphosphonooxy)des-methylCPP) have been developed as probes to measure binding to the NMDA receptor. CGS 39653 (E-2-amino-4-phosphonooxyphosphonomethyl-2-propionic acid) was found to inhibit the binding of [3H]CPP to the NMDA receptor with an IC50 value of 90 µM. The present study examined the characteristics of [3H]CGS 39653 binding to rat forebrain membranes in a filtration assay. Specific binding of [3H]CGS 39653 was saturable, reversible and reached a maximum within 60 min and showed 70-80% of total binding. Computer analysis of binding data from saturation experiments revealed a Kd of 6.2 ± 0.6 nM and a Bmax value of 970 ± 222 fmol/mg protein. When a series of NMDA agonists and antagonists competed with the binding of 2.0 nM [3H]CGS 39653, the following order of potency was generated (Kb): CGS 19755 (43 nM) > L-glutamate (45 nM) > CPP (105 nM) > D-aspartate (1570 nM) > kainate (2700 nM). Quinpirole and kainate inhibited less than 65% specific binding at 10 µM generating steep inhibition curves with Hill slopes near unity. In contrast, glycine produced a biphasic inhibition curve. Computer analysis indicated that 7 ± 1% of the binding was inhibited with an IC50 value of 536 ± 98 nM, whereas 63 ± 1% of the binding was inhibited with an IC50 value of 869 ± 103 µM. These results indicate that [3H]CGS 39653 is a selective, high affinity radioligand for the NMDA receptor.


The pharmacological specificity of excitatory amino acid receptor coupling to brain phosphoinositide hydrolysis was characterized in hippocampal slices from 7 day old rats. Tissue slices were preincubated with 1H-nanosil, washed and the buffer containing 10mM LICI, then incubated with 60 pmol. Antagonists were preincubated 20 min prior to the agonist incubation. Maximal effective concentrations of IBO (10³M) or QUIS (10³ M) increased the formation of 3H-inositol 1,4,5-trisphosphate by about 70%, but 2 or 5 mM NH4Cl reduced it by only about 20%. Whether this depression is pre- or postsynaptic is controversial. To test for a post-synaptic block, firing of units in the pyramidal layer of CA1 area induced by 100 msec pulses of electrophysiological glutamate was tested. Lowering glucose from 5 to 0.2 mM glutamate induced firing by about 70%, but 2 or 5 mM NH4Cl reduced it by only about 20%. If however, 25 µM bicuculline was present, which increased the response of neurons to glutamate, the effectiveness of NH4Cl more than doubled. These observations agree with findings that neither low glucose (Szeber & Ojeda, J. Neurosci. 1:265,1981) nor 5 mM NH4Cl (Butterworth et al. Reciprocal Dopaminopathy, Humana Press, in press) inhibit stimulus-induced release of excitatory amino acids from hippocampal slices and suggest that synaptic transmission is blocked at a post-synaptic site. To reveal the blockade by NH4Cl of glutamate-induced excitation, GABA-ergic inhibition has to be reduced, since, by interfering with the Cl- pump, NH4Cl also decreases this inhibition (Bassane, Brain Res. 210:931,1981). (Supported by the NRC of Canada.)


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Carbetapentane(CB), 3-aminopyridine(CM) and deoxycorticosterone(DM) are centrally-acting antitussives which block spontaneous epileptiform bursting induced by Mg2+-free medium in hippocampal slices. Whether this depression is pre- or postsynaptic is controversial. To test for a post-synaptic block, firing of units in the pyramidal layer of CA1 area induced by 100 msec pulses of electrophysiologic glutamate was tested. Lowering glucose from 5 to 0.2 mM glutamate induced firing by about 70%, but 2 or 5 mM NH4Cl reduced it by only about 20%. Whether this depression is pre- or postsynaptic is controversial. To test for a post-synaptic block, firing of units in the pyramidal layer of CA1 area induced by 100 msec pulses of electrophysiologic glutamate was tested. Lowering glucose from 5 to 0.2 mM glutamate induced firing by about 70%, but 2 or 5 mM NH4Cl reduced it by only about 20%. If however, 25 µM bicuculline was present, which increased the response of neurons to glutamate, the effectiveness of NH4Cl more than doubled. These observations agree with findings that neither low glucose (Szeber & Ojeda, J. Neurosci. 1:265,1981) nor 5 mM NH4Cl (Butterworth et al. Reciprocal Dopaminopathy, Humana Press, in press) inhibit stimulus-induced release of excitatory amino acids from hippocampal slices and suggest that synaptic transmission is blocked at a post-synaptic site. To reveal the blockade by NH4Cl of glutamate-induced excitation, GABA-ergic inhibition has to be reduced, since, by interfering with the Cl- pump, NH4Cl also decreases this inhibition (Bassane, Brain Res. 210:931,1981). (Supported by the NRC of Canada.)

Noncompetitive NMDA antagonist compounds, such as ketamine, are known to preferentially decrease utilization in primary sensory cortices and to shift glucose labeling in terminal afferent fields from layer IV to layer Va (Hamner and Henkenkam, 1990). Selective activation of limbic cortical areas also occurs. We compared the pattern and density of cortical activity following various doses of dl-amino-4-phosphonobutyrate (AP), MK-801, or saline while using combined glucose (CDG) technique.

Arterial and venous cannulas were implanted using halothane anesthesia in male, Sprague-Dawley rats. Four hours later, MK-801 (0.1 or 10.0 mg/kg) or AP (1.5 or 4.0 mmol/kg) were administered and 2DG was given in 15 min thereafter. Computer-assisted analyses of autoradiographs and the brain sections which produced yielded CDG values in different lamination of cortical regions. Histologic analysis allowed precise determination of primary somatosensory (S1), visual (V1), hippocampal and other cortical regions.

This study reports the activity of a structurally novel excitatory amino acid receptor antagonist, LY235053, which was recently isolated from the venom of Conus geographus, a marine gastropod. The compound was evaluated in vitro and in vivo for its ability to antagonize the effects of NMDA receptor agonists. The in vitro studies included competitive binding assays using [3H]-AMPA and [3H]-TCP, as well as electrophysiological recordings in neurons isolated from the rat brain. In vivo studies were conducted using mouse models of NMDA-mediated behaviors. The results demonstrated that LY235053 was a potent and selective NMDA receptor antagonist, with a high degree of affinity and selectivity for the NMDA receptor.


Ascorbic acid (AA) and glutathione (GSH) are naturally occurring compounds that have been shown to have antioxidant properties. In this study, the authors investigated the effects of AA and GSH on the NMDA receptor. They found that both AA and GSH were able to inhibit the binding of [3H]glutamate and reduce the affinity of the receptor, as well as inhibit the effects of NMDA. These findings suggest that AA and GSH may have therapeutic potential in the treatment of NMDA receptor-mediated disorders.

PARADOXICAL EFFECTS OF TYLETAMINE, A POTENT PCP-LIKE AGONIST. J. E. West, J. M. Dahl, and D. L. Wood. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

Tyletamine is a potent PCP-like agonist that has been shown to have various effects on the central nervous system. In this study, the authors examined the effects of tyletamine on the NMDA receptor. They found that tyletamine was able to inhibit the binding of [3H]-TCP and reduce the affinity of the receptor. These findings suggest that tyletamine may have therapeutic potential in the treatment of disorders related to the NMDA receptor.

EXCITATORY AMINO ACIDS: RECEPTORS - IX

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463.19
THE EFFECT OF NONCOMPETITIVE N-METHYL-D-ASPARTATE (NMDA)
ANTAGONISTS ON SELECTED NEUROTRANSMITTER UPTAKE SYSTEMS.
D.K. Boyd and R.G. Davis, Pfizer, Rockville, MD 20852.

Behavioral effects of phencyclidine (PCP) have been attributed to its binding to a site within the NMDA receptor complex, blockade of ion channels, or modulation of neurotransmitter release and/or uptake. While other noncompetitive agents also bind to the PCP site, it is unclear whether it is in various neuronal uptake systems. Using slices from the appropriate rat brain region, a portion of OA (striatum), NE (cortex) and 5-HT (hippocampus), uptake was measured for 5 min., using tritiated ligand at 37°C. PCP was found to have IC50 values of 2.5µM, 0.67µM, and 5.15µM respectively. The other noncompetitive agents showed a wide range of activity with the rank order for each assay being: OA-D: PCP < ifenprodil < MK 801 < dextromethorphan (DM) < ketamine and H-H: DM < ifenprodil < PCP < MK 801 < ketamine. Additionally, the competitive NMDA antagonist, 3-(2-carboxypiperazin-4-yl)propylphosphonic acid (CPP), was inactive in all assays. In conclusion, there is no consistent pattern of activity for uptake inhibition with these agents. Thus, the observed activity may not be responsible for effects common to all non-competitive NMDA antagonists (eg. antischizophrenic efficacy, psychotomimetic side effects).

463.21
EFFECTS OF ALCOHOLS ON THE GLUTAMATERGIC TRANSMISSION.
M.T. Lima-Landman, I.A. Costa, A.C. deicast, I.M. Fonseca, and E.X.

Recently, ethanol (EtOH) has been reported to modify the function of excitatory amino acid receptors (Louvier, D.M. et al, Science, 247:61, 1989; Lima-Landman, M.T. and Albuquerque, E.X., FEBS Lett., 267:1, 1989). The impairment of certain brain function under EtOH intoxication and the growing evidence that links the NMDA- subtype of glutamatergic receptors to the memory and learning processes raised questions about the effects of EtOH actions in the CNS. In the light of these findings, the effects of some aliphatic alcohols were studied on the NMDA and quisqualate-activated single channel currents in Hippocampal pyramidal cells in culture using the patch-clamp technique in outside-out configuration. EtOH (1.74-174 mM) and 0.01% - 1% ketamine decreased the concomitant decrease of mean channel open time. In high concentrations (86.5-174 mM) the Popen decreased with a concomitant decrease of mean channel open time. In quisqualate-activated single channel currents, EtOH (5 mM) decreased the Popen. This effect was enhanced with increasing EtOH concentrations. Mean channel open time of quisqualate-activated single channel currents decreased at low concentrations (1.74-6.55 mM) and the Popen decreased without affecting the mean channel open time; at higher concentrations (86.5-174 mM) the Popen decreased with a concomitant decrease of mean channel open time. In quisqualate-activated single channel currents, EtOH (10 mM) decreased the Popen. This effect was enhanced with increasing EtOH concentrations. Mean channel open time of quisqualate-activated single channel currents decreased only at 500 mM EtOH. All these EtOH effects on NMDA and quisqualate-activated currents could be reversed by washing. Using several alcohols (methanol, 1-butanol and isopentanol) we have observed a differential potency of the alcohol action on different neurotransmitter systems. The other noncompetitive agents showed a wide range of activity with the rank order for each assay being: OA-D: PCP < ifenprodil < MK 801 < dextromethorphan (DM) < ketamine and H-H: DM < ifenprodil < PCP < MK 801 < ketamine. Additionally, the competitive NMDA antagonist, 3-(2-carboxypiperazin-4-yl)propylphosphonic acid (CPP), was inactive in all assays. In conclusion, there is no consistent pattern of activity for uptake inhibition with these agents. Thus, the observed activity may not be responsible for effects common to all non-competitive NMDA antagonists (eg. antischizophrenic efficacy, psychotomimetic side effects).

463.23
CNS BINDING SITES OF THE NOVEL NMDA ANTAGONIST, ARG-636.
E. Meno, M. Guluk, M. Pagnoczi, D. Phillips* and N. Saccomano*.

Pfizer Central Research, Groton, CT 06340.

Argiope aurantia, which blocks NMDA-induced elevations of cGMP in the rat cerebellum noncompetitively and NMDA-stimulated release of [3H]norepinephrine from rat hippocampus, both in vivo and in vitro. Additionally, it antagonizes seizures induced by injection of NMDA in mice (see Seymour and Meno, this meeting). Arg636 can be distinguished from other NMDA antagonists by its unique effects on [3H]Gly binding (see Guluk et al, this meeting). We studied the interaction of [125I]Arg636 with CNS membranes to characterize further the mechanism of action of this novel NMDA antagonist. Both radiolabeled [3H]Gly and [3H]nonradioactive [[3H]Gly were synthesized from Arg636 using a chloromine-T procedure and purified by HPLC. The structure of nonradioactive [[3H]Gly was determined by FAB-MS and NMR techniques to be the C-5-oxo acid derivative. Nonradioactive [[3H]Gly blocked NMDA-induced elevation of cGMP with an IC50 similar to Arg636 (IC50's of 48 and 34 µM, respectively).

[125I]Arg636 bound to rat forebrain membranes with KD and Bmax values of 11.25 µM and 28.95 pmol/mg protein (80% specific). The ability of other known polypeptides and recently discovered polypeptides from Agelenopsis aperta to inhibit its activity as functional neurotransmitter antagonists. No other compounds tested were able to block specific binding. However, divalent cations were potent inhibitors of this binding (IC50's of Mn, Co, Mg and Ca = 0.4-1.3 mM). A similar agonist-like effect was observed with Arg636. [125I]Arg636 binding (see Guluk et al, this meeting) and the effects of divalent cations on [125I]Arg636 binding suggest that these polypeptides may antagonize responses to NMDA by interacting with membrane ion channels.

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463.24
IN VIVO NMDA ANTAGONIST ACTIVITY OF THE POLYAMINE SPIDER VENOM COMPONENT, ARGIOTOXIN-836. P.A. Seymour and N. Meno. Department of Neuroscience, Central Research Division, Pfizer, Inc., Groton, CT 06340.

Argiope aurantia, which blocks NMDA-induced elevations of cGMP in the rat cerebellum noncompetitively and NMDA-stimulated release of [3H]norepinephrine from rat hippocampus, both in vivo and in vitro. Additionally, it antagonizes seizures induced by injection of NMDA in mice (see Seymour and Meno, this meeting). Arg636 can be distinguished from other NMDA antagonists by its unique effects on [3H]Gly binding (see Guluk et al, this meeting). We studied the interaction of [125I]Arg636 with CNS membranes to characterize further the mechanism of action of this novel NMDA antagonist. Both radiolabeled [3H]Gly and [3H]nonradioactive [[3H]Gly were synthesized from Arg636 using a chloromine-T procedure and purified by HPLC. The structure of nonradioactive [[3H]Gly was determined by FAB-MS and NMR techniques to be the C-5-oxo acid derivative. Nonradioactive [[3H]Gly blocked NMDA-induced elevation of cGMP with an IC50 similar to Arg636 (IC50's of 48 and 34 µM, respectively).

[125I]Arg636 bound to rat forebrain membranes with KD and Bmax values of 11.25 µM and 28.95 pmol/mg protein (80% specific). The ability of other known polypeptides and recently discovered polypeptides from Agelenopsis aperta to inhibit its activity as functional neurotransmitter antagonists. No other compounds tested were able to block specific binding. However, divalent cations were potent inhibitors of this binding (IC50's of Mn, Co, Mg and Ca = 0.4-1.3 mM). A similar agonist-like effect was observed with Arg636. [125I]Arg636 binding (see Guluk et al, this meeting) and the effects of divalent cations on [125I]Arg636 binding suggest that these polypeptides may antagonize responses to NMDA by interacting with membrane ion channels.
463.25 POLYAMINE SPIDER TOXINS BLOCK NMDA RECEPTOR-MEDIATED INCREASES IN CYTOSOLIC CALCIUM IN CEREBELLAR GRANULE NEURONS. T. N. Parks1, R. A. Veldman1, H. A. Saccozzi2, L. D. Adman2 and E. F. Nemeth2. Natural Product Sciences Inc., Salt Lake City, UT 84108 and Pfizer Central Research, Groton, CT 06340.

Polyamine-containing toxins in spider venoms have been reported to antagonize transmission at a variety of glutamatergic synapses (Ann. Rev. Neurosci. 12, Am. Rec. Neurol. Chem. 4, 1989). We investigated the effects of synthetic toxins on NMDA-induced changes in cytosolic free calcium concentration ([Ca²⁺]ᵢ) in cultures of neonatal rat cerebellar granule neurons studied by fura-2 fluorometry (Park and Nemeth, this meeting). Increases in [Ca²⁺]ᵢ in response to 10⁻⁵ M NMDA or L-aspartate in these cells are enhanced by 15⁻⁶ M glycine and antagonized by MK-801 (IC₅₀=15 nM), AP-5 (IC₅₀=20 µM) and CPP but not by DNXO. Synthetic polyamine toxins are potent inhibitors of NMDA-induced increases in [Ca²⁺]ᵢ: Arclocxin 659 (IC₅₀= 50 nM); Argioltokin 636 (85 nM); Nefilatokin (NSTX; 370 nM); Argioltokin 601 (500 nM) and E (700 nM); and spider toxin (JSTX; 850 nM); Philantoxokin-433 (15 µM). These data, in conjunction with evidence that polyamine toxins specifically affect NMDA-mediated transmission in rat hippocampus (Mueller et al., this meeting), suggest that these compounds constitute a new class of potent NMDA antagonist (see also Mena et al. and Seymour and Mena, this meeting).

464.1 EFFECTS OF PERIPHERAL ADMINISTRATION OF SUBSTANCE P AND NALOXONE ON AVOIDANCE LEARNING AND HABITUATION. C. Tomas, P. J. C. Viegas and M. I. Aguiar. (SPPR: A. J. Ricardot.) Lab. of Psychobiology, University of Sao Paulo, FCLP-ESP, 14049 Ribeirao Preto, SP, Brazil.

One of us has shown that peripheral post-trial administration of substance P (SP) improves retention test performance of a single-trial inhibitory avoidance task in a dose-dependent way. In the present study, we examined the effects of SP peripheral administration on learning of three avoidance tasks and habituation. Male Wistar rats were tested in three inhibitory avoidance tasks: step-down, up-hill and up-hill and uphill. Habituation was measured in an open field by recording the number of rearings during 2 min of free exploration. Training and test sessions were identical and 24 h apart. The animals were injected i.p. immediately after the training trial with all possible combinations of vehicle, SP (5, 50, 100, 250 or 500 µg/kg) and naloxone (0.5, 5 or 50 mg/kg). Rats injected with 50 µg of SP/kg had significantly longer step-down and up-hill latencies at the retention test than control animals, whereas such differences were not observed for the alcove task. Pre-treatment with naloxone produced facilitation on the up-hill and step-down tasks. In the habituation test, there were significant effects for the doses of 5 and 50 µg SP/kg, lower and higher doses being ineffective. Additionally, for this test, we included a 45 h delayed injection group, which did not differ from the vehicle control. Supported by Brazilian National Research Council (CNPq).


A posttraining administration of opioid antagonists enhances retention in a variety of learning and memory tasks. The role of opioid peptides in spatial tasks, however, is not established. We explored the role of opioid peptides in spatial learning by assessing the effects of pre- and posttraining administration of naloxone on water maze acquisition. Rats were given naloxone HCl (0.1 or 3 mg/kg ip) either 5 min. before or immediately after each day's training session (2 trials/day) for 4 days. A 5th session was conducted without drug injections and was immediately followed by a free swim probe trial. Pretreatment with naloxone produced a dose-dependent increase in the rate of acquisition as measured both by escape latencies during training and search pattern analysis during the free swim. Posttraining naloxone had no significant effect. In a parallel set of rats receiving daily pretraining naloxone (3 mg/kg ip), septohippocampal cholinergic activity, which is thought to be important in the acquisition of spatial information, was assessed by measuring hippocampal HACU 15 min. after the last trial on day 4. HACU was significantly reduced in place-trained rats and in swim-yoked controls relative to behaviorally naive rats. Naloxone significantly reduced HACU in behaviorally naive rats, but attenuated the swimming-induced decrease in HACU. These results suggest that naloxone alters the response of the septohippocampal cholinergic system to water maze training. These experiments suggest that opioid peptides play an important modulatory role in the acquisition of spatial information and that they may produce some of these effects via an action on the septohippocampal cholinergic system. Support: NIA Fellowship AG05446 (MDW), NIMH Grant MH51256 (JLM), ORN Contact N00014-87-K-0518 (JLM).


[Leu]enkephalin (LE) impairs retention of peck-aversion training in two-day-old chicks (Patterson et al., Behav. Neurosci., 103:429, 1989). To characterize further the development of amnesia following intracranial (i.c.) injection of LE, chicks were tested either 5, 15, 30, 45, 60, 90, 120, 180, 240 min, or 24 hr after training. Chicks injected bilaterally in the region of the intermediate medial hypopstriatum ventrale with LE performed like controls when tested at each of the post-training time points between 5 and 180 min. At 240 min post-training, LE-treated chicks show a moderate impairment, and at amnesia when tested at 24 hr. Previous investigation of chicks indicates that amnesia develops within one of three temporal courses. The current findings suggest that injection of LE produces amnesia with novel temporal characteristics. Also, B-enkephalin was found to produce amnesia more rapidly than other agents studied in laces (Bennett, et al., Soc. for Neuroscience, 1988). These results suggest that opioids may affect memory formation through mechanisms other than those previously studied.

464.4 ENHANCEMENT OF MEMORY FOR A ONE-TRIAL PASSIVE AVOIDANCE TASK IN CHICKS GIVEN PERIPHERAL AND CENTRAL INJECTIONS OF NALOXONE. D. W. Lee, M. K. Meaney, D. J. Alkowicz, E. L. Bennett, J. L. Martinez Jr., A. M. Rosenberg, and Department of Psychology, University of California, Berkeley, CA 94720.

To investigate memory enhancement for a one-trial passive avoidance task in chicks, a weak concentration (10%) of the bitter tasting liquid methylanthranilate (MeA) was used as the aversive stimulus. Naloxone (Nal), an opioid receptor antagonist known to generally enhance some forms of memory, was without effect when 100% MeA was used. Several experiments were conducted administering central (i.c.) bilateral injections directed to the region of the medial hypopstriatum ventrale with LE performed like controls when tested at each of the post-training time points between 5 and 180 min. At 240 min post-training, LE-treated chicks show a moderate impairment, and at amnesia when tested at 24 hr. Previous investigation of chicks indicates that amnesia develops within one of three temporal courses. The current findings suggest that injection of LE produces amnesia with novel temporal characteristics. Also, B-enkephalin was found to produce amnesia more rapidly than other agents studied in laces (Bennett, et al., Soc. for Neuroscience, 1988). These results suggest that opioids may affect memory formation through mechanisms other than those previously studied. Supported by NIDA grant DA-04795-02 and NSF grant BNS-88-10528.
464.5 EFFECTS OF POSTNATAL TESTOSTERONE PROPIONATE ADMINISTRATION ON MORRIS WATER MAZE PERFORMANCE IN RATS. R.I. Fenn, Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071.

To study the involvement of testosterone in the development of spatial abilities, intact male and female rats were injected with either 75 ug or 150 ug Testosterone Propionate (TP) or an oil vehicle on days 4 and 6 after birth. At 90+ days of age, the rats were trained and tested on the Morris water maze (MWM). Rats were tested once a day for 6 successive days, and were retested after a 1 week delay. TP effects on MWM performance were observed for the trial at which the first significant drop in time and distance required to reach the platform occurred. TP treated males improved more quickly than control females, and control males improved more quickly than TP treated males. In addition, control males required less time than control females to reach the platform on 4 of the 6 initial trials. There were no differences between the groups on the trials after the delay.

These findings support the hypothesis that steroid hormones have an organizational effect on the developing brain that subsequently influences cognitive functioning.


Models of memory consolidation invoke a multi-stage process wherein the different stages are dependent upon different biochemical mechanisms. Recall at intermediate intervals after associative conditioning may be dependent upon mechanisms involving CAM, whereas longer term responding is thought to involve gene activation and protein synthesis. Using univariate analysis of the proboscis extension reflex of the honey bee, we undertook to determine time windows for sensitivity of recall to different substances that modulate these biochemical mechanisms.

Restricted honey bee workers were conditioned to extend their mouthparts to an odorant by 1 to 4 forward pairings (30 sec ITIs) of that odorant with a 2.0M sugar water reward. All bees were tested with a single extinction trial at one of several times ranging from 5 min to 120 min post-conditioning. In a control series to determine long-term recall levels, responses were equivalent among bees conditioned with 1, 2, 3, or 4 trials at all times through 120 min and involved associative and non-associative behavioral mechanisms.

In several subsequent experimental series, each bee received an abdominal injection containing either saline, or one of several concentrations of forskolin, caffeine, or cycloheximide in the same saline. Bees injected with forskolin 45 min prior to a single conditioning trial had similar levels recall to bees injected with saline alone when tested at either 45 min or 120 min post-conditioning. Bees injected with cycloheximide 30 min prior to conditioning had decreased levels of recall 45 min and 120 min after conditioning with 4 trials. However, effects were mixed in groups injected 120 min prior to conditioning. Biochemical characterization of protein synthesis in these groups of bees may elucidate these phenomena.


The effect of caffeine on memory in women who were and were not taking oral contraceptives was examined. Thirty minutes after ingesting either 0, 2 or 4 mg/kg of caffeine at days 1-5 or days 9-13 of the menstrual cycle, subjects listened to lists of words and provided immediate free recall of each list along with a final free recall of all the lists. Caffeine facilitated recall of words in the primary positions of the lists and women on oral contraceptives recalled fewer primary words than pill-free women. These were also complex interactions between treatment, phase of menstrual cycle, practice and rate of presentation of words. During the final recall, caffeine enhanced overall recall. Caffeine enhanced recall for those on oral contraceptives during the early but not the second phase of the menstrual cycle. These results suggest the need for further study of neuroendocrine modulation of the effects of caffeine.


We have recently reported on the usefulness of a novel water maze for the assessment of learning and memory. From the results of these studies, it was possible to determine by possible effects of the drug or physiological variable on appetite [Kant et al., Pharmacol. Biochem. Behav., 31:487, 1988; Przybelski et al., Biochem. Pharmacol., 37:487, 1988; Przybenski et al., Biochem. Pharmacol., 37:487, 1988]. The maze consists of three sets of four walls arranged in concentric squares set inside a 5 ft. dia. pool. Each of the walls has a door that can be opened or closed providing a variety of possible mazes of varying difficulty. In the present study, two groups of rats (10 rats/group) were trained on one configuration of the water maze. In subsequent testing, one group (B) was always tested on the same "training" maze while the other group (A) was tested on a different maze configuration of approximately the same difficulty each day. Each test day consisted of 3 timed trials begun 15 min following drug injection. Both groups were allowed to rest for 30 sec before each trial. The 3 trials were separated by 50 sec rest periods. Not surprisingly, the B group completed the maze each test day in less time and with fewer errors than did the A group. Investigation of the above results suggests that the B group was discriminated on the identical maze as the B group. Both groups, however, were sensitive to the disruptive effects (increased maze time and/or more errors) of diazepam, amphetamine and caffeine, while atropine did not disrupt maze performance.


C57BL/6 mice perform poorly (Upchurch and Wehner, Beh. Gen. 18: 55, 1988). We have studied parental strain. PKC activities also varied in cortical and hippocampal tissue. Significant correlations between hippocampal phosphatidyl serine-stimulated activity and any learning measure. These data support the conclusion that hippocampal PKC is associated with spatial learning performance as measured by the Morris test. (Supported by NSF BBS-8820074).


Specification of hippocampal cell involvement in memory processes requires a precise description of the manner in which hippocampal cells participate in the coding and retrieval of sensory information. The delayed match to sample (DMTS) paradigm can be used to examine the participation of hippocampal cells in retention of sensory information. We examined the response characteristics of identified hippocampal complex spike cells in a DMTS task in which performance decayed from 80% to 70% mean correct responses over delay intervals ranging from 1:31 sec. Both the temporal and spatial correlates of hippocampal complex spike cell activity were investigated and characterized. Prior studies have shown that delta-9-THC, the psychoactive ingredient in marijuana, disrupts performance at longer but not shorter delays in the DMTS task, closely resembling the effects of hippocampal lesions. Results indicate that 50% of complex spike cells encountered in the dorsal hippocampus responded just after execution of the match response; 56% fired just prior to the sample press and just after the match response, and less than 10% of the cells fired prior to the sample press and just after the match response. THC (2.0 mg/kg, IP) eliminated the response to the sample lever but not the error lever in all cells. THC did not alter the match cell discharge in any group of cells tested. These results suggest that the dissociation of the sample- and match-provoked cell discharges by delta-9-THC was responsible for the significant disruption of behavioral responding in the DMTS task. Possible cellular actions of THC on hippocampal pyramidial cells are described in terms of ongoing studies. [Supported by Grants DA04441, DA04320, and K02 DA0719 to S.A.D.]

Extensive amygdala kindling results in deficits in sleep and in inhibitory avoidance test performance. Neuronal changes in sleep and memory deficits significantly correlated in these animals. The present study shows that administration of either a non-competitive (SA) or competitive (ASA) antagonist of the sleep/behavioral alternation (SA) are also produced by amygdala kindling. These effects correlate with changes in sleep. Glucose, which improves memory in kindled rats, attenuates kindling-induced alternations in SA. Male Sprague-Dawley rats had electrocorticography and bilateral amygdala-kindled. After recovery, 3-h baseline sleep samples were obtained. One group received daily kindling stimulation (bicadicular square waves, 7 ms, 50 kHz) for 2 weeks, while another group served as non-stimulated controls. One week after the last seizure, sleep records were again obtained. All rats were then tested for 30 min ITI. 24 h after the last seizure, glucose (100 mg/kg) or saline injection.

Consistently, kindled rats showed reductions in both number and duration of both paradoxical and nonparadoxical sleep. SA performance was also significantly impaired. Individual differences in several sleep measures were significantly correlated with SA performance in kindled rats. Glucose administration improved SA performance to the level of the non-kindled control group. The results demonstrate relationships between sleep and memory deficits in kindled rats and suggest that glucose might be useful in attenuating some of the sleep-induced memory deficits. (Supported by NIMH 52111, ONH 1100145-85-K4072, and NHSAG 55046).

644.12 NO EVIDENCE FOR TIME-LOCKED EFFECTS OF EMETINE INJECTED INTO THE MHV OF THE CHICK BRAIN. P.A. SERRANO, R.J. RAMUS*, E.L. BENNETT and M.R. ROSENWIG. Department of Psychology, University of California, Berkeley, CA 94720.

Gibbs & Ng (1977) have proposed a three-stage model for the formation of memory in the 2-day-old chick. Each of the three stages, short-term, intermediate-term, and long-term, is characterized by a distinct and separable neural event. The present study examined this model in the formation of memory for an appetitive conditioning task. Both pre- and post-treatment measures were used to examine the acquisition of the memory. The memory task was conditioned using a two-trial passive avoidance task in each 5 min and 20 min post-training performance groups were tested using a two-trial passive avoidance task either 5 min or 20 min after training. Subsequent tests were done at 60, 75, 90, 105 min, and 24 h. The results show that emetine produces amnesia at 90 min in both the 5 min and 20 min post-training injection groups without recovery of the memory. The results suggest that the memory is not an artifact of the time required for the drug to disrupt the memory. Supported by NSF grant BNS-86-00926.


Reversal learning set formation provides a useful model for the study of complex learning in animals. Traditional methods have relied upon single-response instrumental discriminations with their concomitant limitations. Spatial reversal learning set formation was explored in Long-Evans rats using a two-lever automatic assay (AU) procedure, in which one lever (CS+) was paired with food reward and the other (CS-) was not. Reward was provided on a VI 1 trial basis regardless of the responses emitted. Behavior of rats trained on the AU task (n=6) was compared to that of rats trained on a similar instrumental (IN) task (n=7), in which at least one response to the CS+ was rewarded on each trial and both groups acquired the original spatial discrimination (CS+ right, CS- left) at equivalent rates. Both groups formed similar reversal learning sets, requiring fewer trials to reach criterion (DR > 0.9 for 10-trial blocks) across reversal trials. Three of 7 IN rats extinguished during acquisition of R; their performance was reinstated by 2 sessions of remedial training on the AU schedule. Reversal (R) was faster in the IN group on R1, but slower after the learning set had been acquired (R1 to R16). Fitting 2-parameter nonlinear functions to acquisition curves showed that, while asymptotic discrimination accuracy did not differ, rate of acquisition was faster in the AU group (0.13±0.02) than in the IN group (0.05±0.003). These results show that spatial reversal learning sets can be formed in an automatic environment in which reward does not depend upon accurate responding.


Previous studies have indicated that C57BL/6J mice differ from DBA/2J mice in spatial learning ability. In the Morris water task, a test of spatial reference memory. A spatial working memory task in a Y-maze was used to test for the generality of the spatial learning differences between the two strains. Male mice were trained to go down the arms of the Y-maze for a food reward. During a pretraining phase, animals learned that all arms of the maze contained food and that remaining food was not replaced once it was eaten. Following pretraining, mice were trained either in a spatial match-to-sample task (MS) or a spatial nonmatch-to-sample task (NMS) task. The correct response was to turn in the same direction that they had taken during a forced-choice sample trial. This choice took them into a previously unvisited arm after the NMS task, the correct response was to turn down the arm that they had originally started from and that had not contained any food. C57 mice performed better than Dba mice at the MS task and were substantially worse than DBA mice at the NMS task. The results suggest that C57 mice had formed a cognitive map of maze locations where food was likely or unlikely to be. These results are consistent with earlier work on the spatial learning ability of these two strains of mice in the Morris water task.

644.15 LEARNING AND MEMORY IMPAIRMENT IN RATS FED A HIGH SATURATED FAT DIET. G. WINEGARD* and C.E. GREENWOOD (SPON: G.H. ANDERSON). Dept. Psychol., Western University, Vienna, Austria, and Dept. Nutritional Sciences, Univ. Toronto, Toronto, Ont., Canada M5R 3K8.

Our previous studies indicate that alterations in the type of dietary fats fed to rats influence a number of behaviors, including learning and memory performance. To conduct a more systematic investigation of cognitive performance, three groups of male Long-Evans rats (one month of age) were fed 20% (w/w) fat diets high in saturated (SBO)-based) or laboratory chow (4.5% (w/w) fat with similar fatty acid profile to SBO). After three months, all rats were tested on Olton's radial arm maze, a complex learning in animals. Traditional methods have relied upon single-response instrumental discriminations with their concomitant limitations. Spatial reversal learning set formation was explored in Long-Evans rats using a two-lever automatic assay (AU) procedure, in which one lever (CS+) was paired with food reward and the other (CS-) was not. Reward was provided on a VI 1 trial basis regardless of the responses emitted. Behavior of rats trained on the AU task (n=6) was compared to that of rats trained on a similar instrumental (IN) task (n=7), in which at least one response to the CS+ was rewarded on each trial and both groups acquired the original spatial discrimination (CS+ right, CS- left) at equivalent rates. Both groups formed similar reversal learning sets, requiring fewer trials to reach criterion (DR > 0.9 for 10-trial blocks) across reversal trials. Three of 7 IN rats extinguished during acquisition of R; their performance was reinstated by 2 sessions of remedial training on the AU schedule. Reversal (R) was faster in the IN group on R1, but slower after the learning set had been acquired (R1 to R16). Fitting 2-parameter nonlinear functions to acquisition curves showed that, while asymptotic discrimination accuracy did not differ, rate of acquisition was faster in the AU group (0.13±0.02) than in the IN group (0.05±0.003). These results show that spatial reversal learning sets can be formed in an automatic environment in which reward does not depend upon accurate responding.


Upon presentation of a small metal bead dipped in 100% methanol (MeA), a bitter tasting liquid, chicks will peck the bead, taste the bitter liquid, then subsequently avoid a similar but dry bead presented at test. Test can be anytime from 10 to 24 hrs after training. Gibbs & Ng (1977) reported that the initial passive avoidance task shows "dips" (lower percentage avoidance) at about 12 and 55 min posttraining and suggest that these dips mark the transitions between STM & ITM and between ITM & LTM. The existence of a dip in the habituation curve dips has not yet been replicated. Since 80-95% of chicks avoid the bead at most test times, this task may result in a ceiling effect possibly obscuring more subtle aspects of memory formation. Shortened intertrial intervals and moderate peak aversion training of chicks were undertaken both to explore the possibility of occurrence of transitional dips and to conduct studies using memory enhancing drugs.

Two-day-old chicks were injected with 10 µl saline. Five minutes later, they were trained by dipping the bead in either 5%, 10% or 100% MeA. Testing occurred at times from 10 to 24 hrs after training. Diluted MeA results in progressively weaker training and retention: at every test time, chicks trained with the 100% MeA showed greater avoidance (50% at 24 hrs) than those trained with 10% MeA (40%) or those given 5% MeA (32%). Our results also show dips around 1, 15 and 60 min for both the 5% and 10% MeA groups supporting the notion of successive stages in memory formation.

Supported by NSF grant BNS-88-10528 and NIDA grant DA-04795-02.
465.1
EFFECTS OF BUSPIRONE AND ALPRAZOLAM ON A THREE-CHOICE WIN-STAY WATER-ESCAPE WORKING MEMORY TASK IN RATS. E. M. Bass, R. A. McGlenn and J. W. Means, Department of Psychol. & Pharmacol., East Carolina Univ., Greenville, NC 27834.

Two studies were conducted to determine if buspirone (BPS) or alprazolam (ALP) would either improve performance on a water-escape task by reducing perseverative responding or impair performance by interfering with memory processes. Both studies involved giving rats a daily test trial, each consisting of an information run during which guillotine doors forced the rat to enter into the correct escape test run during which the rats could enter any of 3 alleys, but escape only upon entering the same alley to which they had been forced. In the first experiment, rats were trained to a correct choice criterion with 5 min inter-run intervals and then tested for performance with 5, 20 and 60 min inter-run intervals. Rats injected 1 hr before testing with BPS (3mg/kg s.c.) were significantly (p<0.05) impaired relative to rats injected with vehicle, while rats injected with 0.3mg/kg ALP did not differ significantly from controls. In a second experiment, rats in each of the drug groups were tested after receiving 1 of 3 different doses of their respective drug. All three doses of BPS (0.3,1.0mg/kg) impaired performance (p<0.05) while none of the doses of ALP (0.5,1.0mg/kg) impaired performance. Thus, BPS impaired performance of rats on a water-escape working memory task (Supp. by DA-02895).

465.2
CHLOR Diazepoxide and dl-PROPRANOLOL PRETREATMENT OF CONDITIONED HYPERGLYCEMIA. P. S. Crigsson and C. F. Fishbey. Psychology Department, Rutgers University, New Brunswick, NJ 08903.

A history of insulin administration in a novel environment leads to a conditioned hyperglycemic response to water-escape responding in rats when injected with placebo on the test day for conditioning. This hyperglycemic response is reversed to hypoglycemia when pre-treated with chlor diazepoxide (DDP) during both the conditioning and test phases. None of the animals pretreated with chlor diazepoxide (3 mg/kg) showed an increase in plasma glucose concentration on the test day as compared to placebo. Pretreatment with propranolol (5 mg/kg) during the conditioning phase only, on the test day only or during both the conditioning phase and the test day had no effect on the conditioned hyperglycemic response. This tendency was accentuated by propranolol pretreatment. There was no evidence for conditioning in the DDP pretreated groups - possibly due to generalization decrement or state-dependent learning.

465.3
FLUMAZENIL REDUCES POSTOPERATIVE AMNESIA AFTER MIDAZOLAM ANESTHESIA IN AMBULATORY SURGERY PATIENTS. Y. F. Sung, M. D., Co-Investigator: N. Reiss, CRNA*. Department of Anesthesiology, Ambulatory Surgery Center, The Emory Clinic, Atlanta, GA 30322.

Benzodiazepines are known to induce anterograde amnesia - an action which may be desired in certain situations, e.g.: during preoperative and operative manipulation, induction for general anesthesia, and maintenance for general anesthesia. Midazolam hydrochloride has antianxiety and amnestic properties that are similar to those of other benzodiazepines, however, it is unique in that it is water soluble and does not require a solvent that may cause venous irritation during injection. The present report is from a double-blind study performed at a continuous infusion institution to assess the ability of flumazenil, a benzodiazepine antagonist, to reverse the postoperative amnesia after midazolam anesthesia. Midazolam resulted in anterograde amnesia. The patient could not recall picture shown after they received the drug.

In comparison to placebo, flumazenil significantly improved the recall postoperatively after midazolam anesthesia.

465.4

Three experiments were carried out to examine the effects of two benzodiazepines on associative learning as measured by acquisition of the rabbit's nictitating membrane response. In a first study, conditioning was accomplished by pairing light and tone CSs with a shock US. Diazepam (0.1, 0.3 and 1.0 mg/kg) produced a dose-dependent reduction in the acquisition of CSs to both tone and light CSs. The dose of diazepam needed to block criterion acquisition in 50% of the animals was calculated to be 0.33 mg/kg. A second study, employing explicitly unpaired presentations of CSs and US, indicated that diazepam had no effect on baseline responding or on responding to the tone and light CS. Diazepam produced a dose-dependent reduction in the amplitude of the UR elicited by the shock US. In a third study, conditioning was carried out through the pairing of a tone CS and an air puff US. Triazolam (0.05 mg/kg) retarded CR acquisition, while the benzodiazepine antagonist, Ro 15-1788 (3 mg/kg), had no effect. However, Ro 15-1788 completely prevented the retardation in CR acquisition produced by triazolam. It was concluded that diazepam and triazolam were retarding associative learning through an action on the benzodiazepine receptor and that part of this retardation might be due to an effect on the unconditioned reflex. Supported by NIMH Grant MH61641.

465.5
FLUMAZENIL (Ro 15-1788) IMPROVED LEARNING AND MEMORY IN MICE BUT NOT IN MONKEYS. L. Rumennik, G. P. Vincent. was reported to improve learning and memory in mice at monkeys. In an active avoidance procedure, 40 mg/kg, doses ranging from 2.5-40 mg/kg, i.p. (Lai, H. et al., E. Schwam and J. Sepinwall. Department of Neurobiology Atlanta, GA 30322. Ambulatory Surgery Center, The Emory Clinic, New Brunswick, NJ 08903). FLUMAZENIL (Ro 15-1788) IMPROVED LEARNING AND MEMORY IN.

465.6

Diazepam (0.2 mg/kg, administered on eight successive acquisition sessions, impaired a light-cued, successive discrimination in male Sprague-Dawley rats with the animals recovering on three post-drug vehicle sessions. The impairment in discrimination was accompanied by an increase in responding during the four go periods of the task, indicating that DZ-drugged animals have difficulty with holding incorrect responses. The benzodiazepine (BZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the discrimination impairment and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with DZ. These findings suggest that the impairment in DZ-treated rats is mediated by central BZ receptor sites. When administered alone, the 10 mg/kg dose of Ro 15-1788 (but not the 5 mg/kg dose) produced a significant increase in incorrect responses. The benzodiazepine (BZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the discrimination impairment and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with DZ. These findings suggest that the impairment in DZ-treated rats is mediated by central BZ receptor sites. When administered alone, the 10 mg/kg dose of Ro 15-1788 (but not the 5 mg/kg dose) produced a significant increase in incorrect responses.
465.7


The effect of alcohol on learning and memory was assessed in independent groups of student volunteers (total n=28). Subjects were asked to read a number of words that had been typed backwards. Some of these words were from the list that had been imaged and some were new words. Finally, subjects performed a word completion task in which a number of words were presented with letters missing. They were asked to complete each word with the first response that came to mind. The words were either; words that had been imaged; words that had been read backwards; words that had been both imaged and read backwards; or new words. Alcohol caused a dose-related impairment in free recall (p<0.02). In the backwards reading test, both placebo- and alcohol-treated subjects read words faster if they had previously imaged them (p<0.001). In the word completion task, all groups completed more words if they had either read them backwards or imaged them previously (p<0.001). These priming effects did not differ across the groups. The data suggest that alcohol impairs performance in tests of explicit memory but may not impair memory for the same material when tested implicitly.

465.9

VEHICLE INFUSION INTO THE BASAL FOREBRAIN PRODUCES TASK-SPECIFIC COGNITIVE DEFICITS IN THE RAT. J.J. Chrobak, D.A. & T.C. Napier, Department of Pharmacology, Loyola University Medical Center, Maywood, IL 60153.

Basal forebrain nuclei, the ventral pallidum/nucleus basalis (VP/NB) and medial septum (MS), are the source of cholinergic neurons innervated in extrapyramidal processes. Intraseptal infusion of the GABA agonist muscimol produces an impairment in radial arm maze (RAM) performance. The following study addressed this phenomenon within the VP/NB. Rats, trained to perform a RAM task with 1 hr delay between the fourth and fifth arm choice, received vehicle (saline) or Baclofen infused at 100 ng or 500 ng and sham injections into the VP/NB. Vehicle and muscimol infusions, but not sham, produced similar impairments. Deficits persisted for 24 hrs post-infusion. The largest decrement being observed 2 hrs post-infusion. Baseline performance was re-established with further daily testing. A vehicle-induced impairment was not observed in rats tested on a standard RAM task with no delay imposed. Thus, the deficit was dependent upon the mnemonic demands of the task. These findings: 1) indicate that vehicle infusions into the VP/NB can cause a neural perturbation that becomes manifest in particular cognitive testing paradigms; 2) support findings that vehicle infusions into the VP/NB can produce biochemical and behavioral alterations; and, 3) highlight the importance of uninfected controls in protocols that involve treatments within the VP/NB.

465.11

BICUCULLINE METHIODIDE IN THE MRF DOES NOT AFFECT LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE RESPONSE. Wesley P. Jordan and Hilary Donovan*. Psychology. St. Mary's College of Maryland, St. Mary's City, MD 20686.

Short-term habituation of the acoustic startle response in the rat occurs within the response circuit, but long-term habituation is produced by an inhibitory inhibition of the reflex by neural activity in a separate, long-term habituation pathway. The mesencephalic reticular formation (MRF) is a part of the long-term habituation pathway because lesions in this area attenuate long-term habituation of acoustic startle. This study investigated the role of MRF cholinergic (nicotinic-husnory)/acetylcholine (ACh) within the long-term pathway. Drugged rats (n=8) received 50mg of bicuculline methiodide (BMI) to each side of the MRF 10 min before 3 test sessions. Control animals received saline. BMI affected motor activities and depressed startle amplitudes, precluding a clear analysis of habituation. Control rats habituated over sessions. A retest without injections found the deficit to be significant at the end of the 1st session. BMI affected motor activities and decreased startle amplitudes, precluding a clear determination of habituation. Control rats habituated over sessions. A retest without injections found the deficit to be significant at the end of the 1st session. BMI did not impair memory for the same material when tested implicitly.

465.12

DIFFERENTIAL EFFECTS OF BACLOFEN ON ACQUISITION IN POSITIVE VS. NEGATIVE TRANSFER OF DISCRIMINATION TESTS. D.B. Chistose* S. Pena, and M.J. Pontecorvo. CNS Pharmacology, Nova Pharmaceutical Corp., Baltimore, MD 21224.

Rats were trained to criterion in a two-choice, discrete trial auditory/visual discrimination task. Responses on the right lever were reinforced in the presence of a bright light and responses on the left lever were reinforced in the presence of a tone. 24 hours later, and daily thereafter, 1/2 of the rats were injected i.p. with 1.7 mg/kg baclofen, a GABA-A agonist. The other 1/2 received an equal volume of saline. 30 minutes later, rats were trained on a second problem with two new stimuli but with the auditory/visual dimensions constant (i.e., dim light-right, clicker-left). The third problem required responses on the same stimuli but with the auditory/visual dimension reversed (i.e., dim light-right, clicker-left). Baclofen-treated rats required significantly more responses than saline controls to reach criterion for the second problem, but did not require significantly fewer responses to criterion in the third problem. Baclofen did not affect the probability of a response in either problem. Baclofen-induced retrieval impairment could contribute both to the slow learning in Problem 2 (positive transfer task) and to the relatively rapid learning of Problem 3 (negative transfer task). Alternatively, but the long-term habituation asympote of the controls, suggesting that long-term habituation had occurred during the drug sessions. Animals subsequently habituated at a faster rate than the saline controls, but the rate was significantly lower than that observed in the saline controls. This could be at other sites within the pathway.
ALCOHOL- AND BARBITURATE-INDUCED HYPNOSIS FOLLOWING STRESS: S were exposed to 80 escapable or yoked-inescapable to onset of approximately 3 weeks. In contrast, chronic This acute stress exposure did not significantly alter the duration of sleep-time was measured in comparison to naive controls. Both escape and yoked groups did not differ from one another. In Experiment 2, rats were exposed to a more acute stress treatment, 20-5 second with these agents did not exhibit anti-conflict effects at any dose examined. As expected, chronic DMI treatment (5 mg/kg, IP, BID for 5 weeks) did not affect CSB behavior. Finally, chronic TRA treatment (up to 40 mg/kg, IP, BID over 12 weeks) resulted in only a weak anti-conflict effect. These data indicate that not all antidepressant agents exhibit anti-conflict effects when administered chronically. Moreover, the efficacy of these antidepressants to increase punished responding when administered chronically correlates well with their efficacy as anti-panic agents in man. (MH#42501; protocol conforms to NIH guide).

PROTECTIVE EFFECTS OF DM-9384, A PYRROLOID DERIVATIVE, ON CYCLOOXIDASE-INDUCED DECREASE OF GABA AND ACETYLCOLINE RECEPTORS, T. Kitamura*, T. Nakashima, K. Ishida, A. Fujita, M. Taniguchi, H. Matsuura, J. Ichikawa. Dept. of Chem. Pharmacol., Fac. Pharm., Sci., Tohoku Univ., Sendai, Japan. We have reported that N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrroloidinyl)acetamide (DM-9384) ameliorates GABA antagonist and acetylcholine (ACH) induced amnesia in the Wistar rat. Pharmacol. Biochem. Behav. 33: 405, 1989. These results indicate that DM-9384 affects the GABA and AChergic neuronal systems and improves amnesia. In the present experiment, we attempted to investigate whether DM-9384 protects the CM induced decrease in the number of binding sites in GABA and ACh. Two of the 45-study were used for the experiments. Synaptic membranes (PB fraction) were prepared from mouse brain 24 hr after the CM (60 mg/kg, S.C.) treatment. DM-9384 (5 mg/kg, P.O.) was administered 15 min before CM. (H) muscimol and (H) WAY were used as the ligands. Results were as follows: CM significantly decreased bound of (H) muscimol binding sites in the synaptic membranes; DM-9384 protected CM receptors, effect of the effect of CM; CM significantly decreased (H) binding to muscarinic receptors DM-9384 displaced (H) muscimol binding sites from the effect of CM; CM-9384 displaced (H) muscimol binding to the membrane, but not (H) WAY binding. These results suggest that DM-9384 protected the GABA and ACh receptors by binding to GABA, receptor-like binding sites and increasing protein synthesis.

CHRONIC ANTIDEPRESSANT EFFECTS ON CONFLICT BEHAVIOR IN THE RAT. T.J. Hill*, C. Becker*, O.J. Fontana* and A.W. Commissaris, College of Pharmacy, Wayne State University, Detroit, MI 48202. Antidepressant agents vary in their efficacy in the management of panic disorder in man. The present studies examined the effects of chronic post-test treatment with several antidepressants on behavior in the Conditioned Suppression of Drinking (CSD) conflict paradigm. The agents tested were the TCA desipramine (DMI: good anti-panic efficacy), buproprion (BUP: no efficacy) and trazodone (TRAZ: weak efficacy). Acute treatment (IP: 10 min pre-test) with these agents did not exhibit anti-conflict effects at any dose examined. As expected, chronic DMI treatment (5 mg/kg, IP, BID for 5 weeks) resulted in a time-dependent anti-conflict with a latency to onset of approximately 3 weeks. In contrast, chronic BUP treatment (up to 10 mg/kg, IP, BID over 8 weeks) did not affect CSB behavior. Finally, chronic TRA treatment (up to 40 mg/kg, IP, BID over 12 weeks) resulted in only a weak anti-conflict effect. These data indicate that not all antidepressant agents exhibit anti-conflict effects when administered chronically. Moreover, the efficacy of these antidepressants to increase punished responding when administered chronically correlates well with their efficacy as anti-panic agents in man. (MH#42501; protocol conforms to NIH guide).

BEHAVIORAL PHARMACOLOGY: OTHER

ALCOHOL- AND BARBITURATE-INDUCED HYPNOSIS FOLLOWING STRESS: CONTROLLABILITY AND DURATION ARE CRITICAL, DEPENDENCY. R.C. Drugan, D.M. Schorr*, V. Sarabanchuy, & P. Holmes, Dept. of Psychology, Brown University, Providence, R.I. 02912 The two experiments were conducted to examine the impact of controllability and duration of shock on both barbiturate- and alcohol-induced hypnosis in rats. In Experiment 1, rats were exposed to either 80 escapable or yoked-inescapable shocks. Immediately or 2 hours later, all rats were injected with a hypnotic dose of ethambutol pentobarbital or ethanol and the duration of sleep-time was measured in comparison to naive controls. Both escape and yoked groups showed a significantly longer barbiturate-induced sleep-time when compared to naive controls. However, ethanol-induced hypnosis was differentially altered by the controllability of stress. Only the inescapably-shocked group demonstrated decreased ethanol-induced sleep-time in comparison to both escapably-shocked and naive controls, which did not differ from one another. In Experiment 2, rats were exposed to a more acute stress treatment, 20-5 second inescapable shocks, and were injected with either sodium pentobarbital or ethanol immediately or 2 hours post-stress. This acute stress exposure did not significantly alter the barbiturate or ethanol-induced hypnosis at either time point. These results suggest that controllability and duration of stress can markedly influence the hypnotic potency of 2 classes of central nervous system (CNS) depressants.
646.5


The behavioral profile of the conditioned locomotor response (CLR) produced by MDMA was compared with that of the Behavioral Potential (BP) system. The BP provides detailed information regarding amount and qualitative patterning of locomotor activity and investigatory responses. Two injection-free baseline days were followed by drug testing days. The conditioned group was injected with MDMA 5 mg/kg i.p. immediately prior to placement in the BP chambers for a 60 min session and saline upon return to the home cages. The pseudo-conditioned group was injected in the reverse sequence and the control group was injected with saline at both times. On the test day, all rats were injected with saline and tested for a CLR.

The unconditioned profile of MDMA consisted of increased crosses (horizontal locomotion), decreased rears and control levels of holsteps, whereas, the conditioned profile included increased crosses concomitant with increased holsteps. The conditioned increase in locomotion was determined to last at least 3 days under extinction parameters. The rats were released 19 days after the last extinction trial and compared with a naive group of rats. Relative to the control group and pseudo-conditioned group, the conditioned group exhibited increased crosses and the naive group exhibited increased crosses, holsteps and rears at this time. Furthermore, examination of the spatial representations of rats' locomotor-plots on various days will contribute to the evaluation of the behavioral qualities of the CLR produced by MDMA.

(Supported by NIDA grants DA 0333, DA 0498 and DA 02925)

646.6

PHENOFEN ANTAGONIZES THE ANTIINOCICEPTIVE AND SE- DATIVE EFFECTS OF (-)BACLOFEN. C.D. Luce and M. Marcelli. Department of Pharmacology and Toxicology, Research Institute of Scripps Clinic and UCSD Dept. of Psychiatry, La Jolla, CA 92037.

Baclofen (BAC), a selective GABA-B agonist, induces myorelaxant, sedative and antinociceptive effects. In rats, (-)baclofen delayed the hot-tail-flick (ED50 = 4.1 mg/kg ip) and hot-plate (ED50 = 3.2 mg/kg ip) tests. These effects are antagonized by ketanserin (3.0-10.0 mg/kg, ip). Administration of (-)baclofen antagonized the antinociceptive and sedative effects of (-)baclofen. In mice, the antinociceptive effect of (-)baclofen is counteracted by phencyclidine (PCP) (12.5-50 µg icv), a phencyclidine derivative of BAC (Gili- anis, O. E., Jur. Pharm., 154:225,1988).

In rats, pretreatment (1 min) with PHA icv (100 mg/kg) but not systemically (50 mg/kg icp) counteracts the responses of (-)baclofen. A competitive antagonism is observed in tail-flick (ED50 = 6.9 mg/kg) and hot-plate (ED50 = 6.8 mg/kg) tests. The EEG pattern is desynchronized; 2) the righting reflex is absent.

These data indicate that GABA-B, sensitive receptors mediate the sedative and antinociceptive effects of (-)baclofen in rats.

(Supported by NIAA grant #80-11-290)

646.7


Intraperitoneally administered dopamine (DA) agonists and antagonists as well as microinjections of muscimol (GABA agonist) and bicuculline (GABA antagonist) into the substantia nigra reticulata (SNr), a major striatal efferent target, were studied for their effects on different parameters of the reaction time (RT) response in the rat. Animals were shaped to release a lever in response to an auditory/visual stimulus in order to conduct a foraging task. Parameters of primary interest are percent successful avoidance (% avoidance), the latency (speed) of the successful response, and response consistency. The % avoidance as well as the successful latencies determined by DA antagonists (haloperidol) were decreased by approximately 60 and 50% with 100 µg/kg SC 23390 (SCH) and haloperidol (HAP), respectively. The response latencies were increased by about 65 (ms) and 40% (130 ms) in SCH and HAL treated animals, respectively. The selective D2 antagonist antipsychotic (1.0 and 10 µg/kg ip) produced similar though less pronounced effects. Microinjections of BIC (10 and 50 ng/5 µl) into the SNr produced a similar pattern of disruption, decreasing % avoidance by about 47% and increasing the successful latencies by about 45% (130 ms) at the high dose. This pattern of disruption was not observed with the direct acting antagonist apomorphine (APO) or with MUS microinjections (5 and 25 ng/5 µl). Apomorphine produced a decrease in % avoidance similar to that of SCH (=47%) but the response latencies were not affected. Similarly, microinjections of MUS into the SNr decreased % avoidance (=40%) but had little effect on the response latencies. Amphetamine had no effect on % avoidance while slightly decreasing response latencies at the doses used in these studies (0.1, 0.2, and 1.0 mg/kg). These results suggest that different parameters of the RT response are sensitive to systemically administered DA agonists and antagonists in a manner consistent with the effects of GABA agonists and antagonists in the SNr. (Supported by NS 28027 and MH 114779)

646.8

CHRONIC HINDLIMB FLEXION IN RAT IS SUPPRESSED BY 5-HT2 AGONIST, DOI, AND RESTORED BY 5-HT2 ANTAGONIST, KETAMERIN. R. G. Anderson*, D.J. Mokl·er and B.J. Winterson. Dept of Physiologhy and Pharmacology, University of New England College of Osteopathic Medicine, Biddeford, ME 04005.

Electrical stimulation for 1 sec across a rat hindlimb induced an ipsilateral flexor response at a peripheral intensity of 8.8 ± 3.9 g. Spinal section at the mid-thoracic level resulted in a significant increase (rebound) in flexion (=13.0 g). Previously, we reported that either depletion of central 5-HT stores or non-selective 5-HT antagonism eliminated rebound. Presently, (=)-1-(2,5-Dimethoxy-4-iodophenyl)-2aminopropane (DOI) was administered after spinal section and the effect of cumulative doses assessed. Flexion averaged 5.7 g at 72h and increased to 9.3 g with spinal section. Between 3.0 and 10.0 mg/kg, DOI increased flexion. DOI dose-dependently suppressed (=8.2 g). Subsequent administration of ketanserin (3.0-10.0 mg/kg, i.p.) resulted in a % inhibition of DOI recovery. Also, the selective 5-HT2 agonist, (2)-1-(3-(Trifluoromethyl) phenyl)piperazinone (TIPMP), was administered in cumulative doses (0.1-10.0 mg/kg, i.p.) prior to spinal section. No dose produced a change in flexion (=8.4 g). However, the amount of rebound was significantly reduced in comparison to controls (.2 vs 4.7 g). These results suggest that supraspinal inhibition is modulated by the combined effects of postsynaptic 5-HT1 and presynaptic 5-HT2 receptor stimulation. (Supported by NIAA grant #80-11-290)

646.9


Male rats were trained to discriminate intraperitoncularly administered 2.0 mg fenfluramine (FEN) from the vehicle using a two-lever, two-motivated operand discrimination task. Once trained, the rats showed a dose-dependent decrease in discriminative performance on the FEN-incorrect lever. Tested 20 min following decreasing doses of FEN (ED50 = 1.09 µg/kg), Administration of 2.0 µg/kg norfenfluramine (NF) 20 min prior to testing produced 94% responding on the FEN-incorrect lever. The 2.0 µg/kg dose of both FEN and NF was tested at post-administration times ranging from 5 min to 1640 min after administration. NF was observed to have a more rapid onset and a longer duration of action in the FEN-trained rats. These time-course results confirm previous work in this laboratory (Pharmacology Biochemistry & Behavior 33:305-11, 1988). Using rats trained to discriminate NF and FEN, however, the difference in onset and offset of action suggests a possible difference in the parent drug and its metabolite.

This work was supported by NIDA grant No. 04181.

(Supported by NIDA grants DA 0333, DA 0498 and DA 02925)

646.10


Weiss and colleagues have initiated a breeding program to generate strains of rats which display either high or low motor activity (H & L, respectively) in a swim test. After four generations of selective breeding, H and L rats were subjected to: 1) inescapable shock (3 hr randomly distributed days). Rats were tested 20 min following decreasing doses of FEN (ED50 = 1.09 µg/kg) or saline. NF was observed to have a more rapid onset and a longer duration of action in the FEN-trained rats. These time-course results confirm previous work in this laboratory (Pharmacology Biochemistry & Behavior 33:305-11, 1988). Using rats trained to discriminate NF and FEN. However, the difference in onset and offset of action suggests a possible difference in the parent drug and its metabolite.

This work was supported by NIDA grant No. 04181.

(Supported by the Vet. Adm. & USPHS grant #466.9)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

A selective breeding program was undertaken to generate populations of rats which display either high or low motor activity in a swim test. Eighty-two male and 42 female Sprague-Dawley rats were screened in a 15 minute swim test in which the total time spent struggling and floating was measured. The most active and least active males and females were then identified and bred. This screening and breeding process has been repeated for five generations producing rats that differ markedly in the swim test.

Fourth generation Highs and Lows were examined on a number of measures (see also accompanying abstract). Electrophysiologically, locus coeruleus (LC) neurons of Lows displayed higher spontaneous and evoked activity than those of Highs. This pattern of LC activity in Lows resembles that seen in normal animals following pharmacological blockade of α-2 receptors. Consistent with these electrophysiological results, spontaneous injection of the α-2 blocker yohimbine decreased swim test struggling in Highs, while the adrenergic diazepam, which has been shown to increase LC responsiveness, increased swim test struggling in Lows. These results suggest that the behavioral differences observed between these rat lines are mediated through alterations in LC functioning.


The β-adrenergic receptor blocker timolol, was microinjected into the gigantocellular tegmental field (FTG) and its pharmacologic effect on rapid eye movement (REM) sleep studied in 3 unanesthetized adult male cats. Our protocol consisted of 4 baseline control polygraphic recordings for each cat (n=12) followed by 24 injection trials with timolol (4µg/250nl) at 6 pontine sites (2.39, 1.61, 3.79). Results indicate a 300% increase in the amount of time associated with PGO wave activity (p<0.001) compared to controls, and an enhancement of REM sleep behavior. Mean REM sleep latency (29.8±5.3 min.) and PGO Latency (12.8±5.5 min.) were significantly shortened (p<0.01) compared to controls. The ED50 vs motor activity is 12.8 mg/kg ip, whereas against electroshock-, pentyleneetetrazol-, and 3-mercaptopropionic acid-induced seizures was 12.8 mg/kg ip, whereas against electroshock-, pentyleneetetrazol-, and 3-mercaptopropionic acid-induced seizures, ED50 values were 350 µg/kg. CNS depressant effects are minimal, since the ED50 vs motor activity is >200 mg/kg, and the LD50 is >800 mg/kg ip.


MDL 27531 ([8-1,2,4-triazole,4-methyl-3-(methylsulfonyl)-5-phenyl-] is a specific antagonist of strychnine-induced seizures in mice relative to its other seizure tests. Thus, its ED50 against strychnine-induced seizures was 12.8 mg/kg ip, whereas against electroshock-, pentyleneetetrazol-, and 3-mercaptopropionic acid-induced seizures, ED50 values were 350 µg/kg. CNS depressant effects are minimal, since the ED50 vs motor activity is >200 mg/kg, and the LD50 is >800 mg/kg ip. Many known muscle relaxants are moderately effective against strychnine-induced seizures in mice, but cause CNS depressant activity at effective doses.

This profile was generally reflected in a rat model in which MDL 27531 selectively attenuated the excitatory effect of a subconvulsant dose (0.9 mg/kg ip) of strychnine on acoustic or tactile startle reflexes. MDL 27531 did not depress baseline and did not antagonize the enhanced reflexes produced by rolipram (0.5 mg/kg ip) or d-amphetamine (4.0 mg/kg ip). In contrast, diazepam (5.0 mg/kg ip) or gamma-vinyl-GABA (1.9 g/kg ip) markedly depressed startle baseline but did not diminish strychnine excitation.

It is suggested that MDL 27531 may be a novel centrally-acting muscle relaxant with minimal CNS depressant activity.
L- AND D-BA Gol FEN INDUCE DIFFERENT CARDIOVASCULAR RESPONSES IN THE RAT AFTER INTRATHecal ADMINISTRATION. L. Huang and J. L. Breyer (SPON: S.N. Young) Depts of Physiology and Psychi atr i c s , McGill Univ, Montr e al, H3x 1Y6. Our pr evious results ind icated that bioscience-sensitive GABA_ receptors in the rat spinal cord are involved in regulation of arterial pressure and heart rate (Doc. N e u r o s c i . A b st. 14:190). The purpose of the present study was to examine the possible role of GABA_A receptors by determining the effects of L- and D-baclofen given intrathecally at the T2 and T9 spinal levels in urethane (2.5 g/kg, i.p.)-induced a dose-dependent increase in arterial pressure without altering heart rate. The present study was to examine the possible role of GABA_A receptors by determining the effects of L- and D-baclofen given intrathecally at the T2 spinal level. Baclofen (200 pmol/side), a GABA-Aergic agent, caused a marked increase in arterial pressure without altering heart rate; the effects were abolished by pretreatment with (i) hexamethonium (10 mg/kg, i.v., n = 7) and (ii) lido caine (2% solution, intrathecal, n = 7). Control rats, given CSF only, exhibited no responses to injection. The results suggest that L- and D-baclofen-sensitive GABA_A receptors in the spinal cord are involved in regulation of spinal sympathetic output controlling arterial pressure and/or heart rate. 

(Supported by a grant from the Quebec Heart Foundation to JLH)

GABA ACTS ON GABA_SA RECEPTORS IN NUCLEUS TRACTUS SOLITARIUS TO INCREASE BLOOD PRESSURE. J.C. Sved and A.F. Sved. Department of Behavioral Neurosciences, University of Pittsburgh, Pennsylvania, PA 15260. Local injection of nipeptonic acid (NIP), an inhibitor of GABA uptake, into the nucleus tractus solitarius (NTS) of chloralose-anesthetized rats increases arterial pressure (AP) presumably by potentiating the action of endogenously released GABA. Although agonists of a GABA receptor increased AP when administered into the NTS, the pressor response to NIP is not antagonized by drugs that block the GABA_A receptor. The present study examines the effects of a newly described inhibitor of the GABA-A receptor, phaclofen (PHAC). PHAC (4 nmol in 10 nl artificial CSF) administered bilaterally into the NTS slightly decreased AP (-82 +/- 42 mmHg, n=6, P<0.05) while heart rate did not change. Higher doses were not tested due to the drug's limited solubility and lower doses did not significantly change AP. PHAC (4 nmol) reversed the pressor response to NIP (10 nmol) injected into the NTS. PHAC (0.1-4 nmol) administered into the NTS antagonized in a dose dependent manner the pressor effect of in NTS injections of baclofen (1-100 pmol), a specific GABA_A agonist. PHAC did not alter the pressor response to injection into the NTS of the selective GABA-A antagonist muscimol. These results demonstrate that the pressor response to NIP injected into the NTS is mediated via GABA_A receptors, suggesting that the NTS endogenous GABA influences cardiovascular function predominantly through an action on GABA_A receptors.

467.3

THE EFFECTS OF INTRACEREBROVENTRICULARLY INFUSED GABA IN SPONTANEOUSLY HYPERTENSIVE AND NORMAL RATS. Kim A. Roberts, Joseph Hardin, and John W. Wright Washington State University, Pullman, WA, 99164. Intracerebroventricular (iv) infusion of GABA at various doses was investigated in Spontaneously Hypertensive rats (SHRs), and Wistar-Kyoto (WKY), and Sprague Dawley (SD) normotensive rats. Previous investigations have reported that ivc injections of GABA, or muscimol the potent GABA agonist, produced hypotension in rats (Perron, 1989). In our experiments GABA was infused at doses of 0, 100, 1,000 and 10,000 nmol/min. Mean arterial pressure (MAP) and heart rate (HR) were recorded at 5 min intervals. The maximum decrease in MAP was 34 mmHg and required 50 min for recovery. Our findings agree with earlier reports suggesting that ivc injections of GABA significantly alter blood pressure (Unger et al., 1984); however, by utilizing three strains of rats and administration into the 4th cerebral ventricle, we wish to demonstrate that ivc injections of GABA decrease MAP from basel ince in a dose-dependent manner in members of all strains, with the SHR exhibiting the greatest decrease in basal MAP. GABA was given in a dose 100,000fold larger than intrathecal injections of GABA. Supported by NIH grants HL32063 and TW11112 and the American Heart Association.


HR (beats/min) BP (mmHg)

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+ change from BASAL by paired t-test, p<0.05

These results suggest that stimulation of GABA_A receptors in the PH blocks the HR response to experimental stress in rats. (Supported by USPHS grant NS 89833)


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+ change from BASAL by paired t-test, p<0.05

These results suggest that stimulation of GABA_A receptors in the PH blocks the HR response to experimental stress in rats. (Supported by USPHS grant NS 89833)

CARDIORESPIRATORY EFFECTS CAUSED BY MICROINJECTION OF NMDA OR KAINIC ACID (KA) INTO THE POSTERIOR HYPOThALAMUS (PH) IN ANESTHETIZED RATS. A.F. Sved* and J.L. Breyer* (SPON: G. Nicol). Dept. of Pharmacol. & Toxicol., Univ. Sch. of Med., Indianapolis, IN 46202-5120. Drugs which interfere with the inhibitory function of the neurotransmitter gamma aminobutyric acid (GABA) act within the PH to produce cardiovascular, behavioral, and respiratory changes resembling those seen in stress in rats. This study examined the hypothesis that injection of excitatory amino acid receptor agonists into the PH would cause similar effects in urethane-anesthetized rats. NMDA or KA was microinjected in 50 nL of saline into the same site in the PH where GABA antagonist bicuculline methiodide 10 ng produced chemotaxis (70 beats/min), mean arterial pressure (MAP: 143±4* mmHg), and respiratory rate (RR: 29±3 breaths/min). Below an approximate dose of 50 nL of saline (RR: 29±3 breaths/min), mean arterial pressure (MAP: 143±4* mmHg), and respiratory rate (RR: 29±3 breaths/min). Below an approximate dose of 50 nL of saline (RR: 29±3 breaths/min), mean arterial pressure (MAP: 143±4* mmHg), and respiratory rate (RR: 29±3 breaths/min). Below an approximate dose of 50 nL of saline (RR: 29±3 breaths/min). Below an approximate dose of 50 nL of saline (RR: 29±3 breaths/min). Below an approximate dose of 50 nL of saline (RR: 29±3 breaths/min).
INHIBITION OF THE ARTERIAL BAROREFLEX BY A SPECIFIC N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST. T. H. Guard and B. R. Felder. Dept. of Int. Med. and CV Center, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Recent studies have suggested that N-methyl-D-aspartate (NMDA) receptors are involved in baroreflex regulation. We tested the effects of the specific non-competitive NMDA antagonist (−)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine maleate (MK-801) on baroreflex control of sympathetic neural activity. Invasive. Renal (ESNA, n=5) and cardiac (CSNA, n=4) sympathetic nerve activities were measured in 3 chloralose anesthetized cats before and after intravenous injection of MK-801 (1 mg/kg). The baroreflex was assessed during ramp increases in arterial pressure over the range of 60–200 mmHg. MS-01 administration did not change mean arterial pressure (MAP) but decreased both the maximum gain (Gmax) and the range (R) of inhibition of the MAP vs. SNA relation. (* significantly different from Control (C), p<0.01).

Gmax (%/mmHg) R (% MAP)
60 min 60 min
ESNA 1.02±0.3 0.23±0.1 17±7.5 7.7±2.1
CSNA 1.21±0.3 0.13±0.1 65.5±16 8.4±1.7

Inhibition of the baroreflex was evident at 10 min and remained suppressed at 3 hours following MK-801 administration. These data are consistent with the view that central excitatory amino acid pathways acting on NMDA receptors mediate the arterial baroreflex.
467.13

The one-kidney-Goldblatt (1-K-G) model of cardiovascular hypertension alters thermal tolerance, and central catecholaminergic processes, including those of the PVN. The PVN contains a variety of neurons that project to preganglionic cell bodies controlling thermogenesis. We tested the effects of micro-injection of clonidine (Clon) into the ipsilateral PVN in 1-K-G rats in conscious and anesthetized states. Clon dose-dependently increased and decreased body temperature in conscious and anesthetized rats, respectively. In anesthetized rats, Clon decreased body temperature in a dose-dependent manner, with an effective dose of 1.0 μg. Clon also decreased BP in a dose-dependent manner, with an effective dose of 1.0 μg. These results suggest that Clon may have a potential therapeutic effect on hyperthermia and hypertension.

467.15
SEPARATE MECHANISMS BY WHICH CLONIDINE DEPRESSES INTRASPINAL AND GANGLIONIC SYNAPTIC TRANSMISSION. Donald N. Franz, Scott C. Steffen*, and Lewis C. Miner*, Dept. of Physiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5T9.

This study investigated the mechanisms by which clonidine (Clon, 5-50 μg/kg) depresses sympathetic preganglionic neurons (SPGNs) following intraspinal Clon injections in a rat lumbar sympathetic spinal ganglion. Clon depressed the spontaneous activity of SPGNs in a dose-dependent manner, with an effective dose of 5 μg/kg. Clon also decreased the BP in a dose-dependent manner, with an effective dose of 5 μg/kg. These results suggest that Clon may have a potential therapeutic effect on hyperthermia and hypertension.

467.17

A map of A1 noradrenergic projections to the nucleus of the solitary tract (NTS) in the cat was obtained using the retrograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) and the anterograde tracer biotinylated dextran amine (BD-DA). PHA-L labelled cells were seen in the region of the A1 noradrenergic cell group projecting to the NTS. BD-DA labelled fibers and terminals were observed in the NTS. These projections were dose-dependently increased in a dose-dependent manner, with an effective dose of 1.0 μg/kg. These results suggest that the A1 noradrenergic cell group may have a potential therapeutic effect on hyperthermia and hypertension.

467.14

Clonidine-dispersing substance (CDS) is a low-MW substance in brain identified by displacement of [125I]-Clon from specific cerebral cortical receptors. We tested the effects of micro-injection of Clon into the PVN in anesthetized rats and observed the changes in BP and rectal temperature (Tr) during H were significantly higher in the control group (n=7) than in the Clon-treated group (n=7). These results suggest that Clon may have a potential therapeutic effect on hyperthermia and hypertension.

467.16

The acute effects of osyverazolazide (OXY:2 μg/kg) and iodoxazol (IDZ:200 μg/kg) on BP, rectal temperature (Tr), and the release of NE and NPY were studied. OXY significantly decreased BP and Tr, while IDZ significantly increased BP and Tr. These results suggest that OXY and IDZ may have a potential therapeutic effect on hyperthermia and hypertension.

467.18
EFFECTS OF CENTRAL ADMINISTRATION OF 8-OH-DPAT ON AUTONOMIC OUTLOW IN ANESTHETIZED CATS. A. G. Ramage, E. J. Shoebread, and D. Jordan, Departments of Pharmacology & Physiology, Royal Frees Hospital Medical School, Rowland Hill Street, London NW3 2PP, England.

8-OH-DPAT, a 5-HT1A agonist and 8-OH-DPAT, when given i.o., evoked a centrally mediated vasomotor and sympatho-inhibition (Ramage, A. G., & Pozzard, J. R., Eur. J. Pharmacol. 138:179, 1987). The present study investigated the effects of 8-OH-DPAT on BP and heart rate in anesthetized cats. 8-OH-DPAT significantly decreased BP and heart rate. These results suggest that 8-OH-DPAT may have a potential therapeutic effect on hyperthermia and hypertension.

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THURSDAY PM
CARDIOVASCULAR REGULATION V
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POWER SPECTRAL ANALYSIS OF HEART RATE VARIABILITY IN PROPRANOLOL TREATED RATS. W.E. Rote* and J.D. Connor. Dept. of Anatomy & Cell Biology & Brain Res. Inst., UCLA, Los Angeles, CA 90024.

We studied two groups of methoxyflurane anesthetized rats (male, S-D, wt=255-455g); saline infused (S, n=6) and propranolol infused (P, 4mg/kg/hr, n=6) to determine the utility of PSA. Pre-infusion heart rate (HR) and power spectral densities in P and S groups were S=376.5±10.4 SE and P=351.7±6.4. During 1st infusion, mean integrated HR was 468.2±12.3, while P decreased HR to 468.3±10.4 (p<0.05). Power spectral analysis of HRV during S infusion showed a slight decrease in both the low and high frequency power and an increase in the mid frequency range (LOW=3.1±0.9; MID=+9.8±2.2; 0.01-0.10 Hz, MID=+11.4±2.1). This indicates reduced sympathetic activity. The P infusion caused a large decrease in power spectral density at the low frequency range (LOW=3.1±0.9; 0.01-0.10 Hz, MID=+11.4±2.1). This indicates reduced sympathetic activity.

We directly measured renal sympathetic nerve activity (RNA) during static exercise in awake cats. After the cats were trained to perform bar-presses for 1 min using a forelimb, they were instrumented for recording RNA, arterial pressure (AP) and heart rate (HR). The exercise experiments were conducted 3-10 days after surgery. RNA, AP, and HR increased during static exercise. The increase in RNA had both an initial and a late component. The initial increase in RNA occurred simultaneously with the onset of exercise and the late increase in RNA was progressively developed 20-30 sec after the onset of exercise. AP increased in parallel with the renal nerve response. To examine any influence of the arterial baroreflex on the RNA responses to static exercise, we allowed the cats to perform static exercise when AP was changed by infusion of norepinephrine or nitroprusside. The increase in RNA in response to static exercise was almost abolished when resting AP was increased; whereas, it was enhanced when resting AP was decreased. Thus, we suggest that the abrupt increase in RNA during static exercise is evoked by central activation of the sympathetic nervous system and that the arterial baroreflex significantly influences RNA during exercise.

Our previous studies have shown that anesthetization of rostral ventrolateral medulla (RVLM) and rostral ventromedial medulla (VLM) with the local anesthetic lidocaine (LIDO) attenuates the pressor response produced by microinjected in CVMM produced a significant depressor response with no change in HR. The pressor response produced by ICV administration of 200 ng ANG-II was not altered. Saline administration had no effect on the above cardiovascular parameters. This study was supported by NIH grant NS 26147.

POSITIVE BRAINSTEM SITES INVOLVED IN THE MEDIATION OF THE CENTRAL ANGIOTENSIN II (ANG-II) PRESSOR RESPONSE. L.R. Portis and J.A. Fisher. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Prior studies demonstrated that microinjections of opioids into the dorsal vagal complex (DVC) elicited cardiovascular responses, which were a function of receptor subtype and injection site. In the present study regional blood flow was measured with pulsed Doppler flowprobes and with radioactive microspheres following injection of small volumes (10 nl) of selective mu or kappa receptor ligands into the DVC. All experiments were performed on male Sprague-Dawley rats, anesthetized with pentobarbital, paralyzed with d-tubocurarine and artificially ventilated. Following mu receptor activation in the DVC, mean blood pressure decreased 10-30 mmHg, while kappa receptor activation in the DVC elicited an increase in mean blood pressure of 10-30 mmHg. These data suggest that mu receptor activation in the DVC elicits a depressor response, while kappa receptor activation in the DVC elicits apressor response. Further research is needed to clarify the role of opioid receptors in cardiovascular regulation.
468.11 CENTRAL NERVOUS SYSTEM CARDIOVASCULAR ACTIONS OF CORTICOTROPIN-RELAXING FACTOR: EFFECTS OF ANGIOTENSIN II ON HYPERALGESIA. C. Fisher, G. Gurney, and J.M. O’Gorman. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Corticotropic-releasing factor (CRF) acts on the central nervous system (CNS) to increase sympathetic nervous activity, decrease parasympathetic nervous activity, elevate arterial pressure (AP) and increase heart rate (HR). Moreover, CRF within the CNS produces behavioral activation indicative of enhanced fear. By virtue of its CNS distribution and actions, CRF is hypothesized to be a key physiologic mediator of the endocrine, autonomic and behavioral responses to stress. The anxiolytic agents, diazepam and alprazolam, are reported to suppress activation of sympathetic nervous outflow. The present studies were aimed at determining whether these drugs suppressed induced sympathetic and cardiovascular activation. Conscious, unrestrained male Sprague-Dawley rats fitted with indwelling intracerebroventricular (icv) cannulae and iliac arterial catheters were used in all experiments. Rats received intraperitoneal injections of vehicle (1 ml/kg), diazepam (1-5 mg/kg) or alprazolam (0.5-3 mg/kg) thirty minutes prior to icv administration of saline (10 µl) or CRF (0.15 nmol). In rats receiving saline icv, diazepam and alprazolam treatments produced small elevations of baseline HR without affecting baseline AP. CRF-induced elevations of AP were attenuated after pretreatment with diazepam and alprazolam. In contrast, CRF-induced elevations of HR were not affected and were slightly enhanced by diazepam and alprazolam pretreatments. These data suggest that CNS-mediated cardiovascular activation by CRF is subject to modulation by benzodiazepine anxiolytics.

468.13 EFFECT OF NALOXONE INJECTIONS INTO BRAINSTEM NUCLEI ON BAROREFLEX IN MORPHINE-TREATED RATS. Karen L. Cochrane and Patrice G. Guvenet. Department of Pharmacology, University of Virginia, Charlottesville, VA 22908.

Baroreflex slope (BRS, delta SNA per mmHg) was assessed before and after iv morphine (MOR: 8mg/kg) and after naloxone (NAL: 4µg) into the caudal (CVL) and rostral (RVL) ventrolateral medulla and rostral ventro lateral medulla (RVL) in rats anesthetized with halothane. The min and max levels of SNA obtained after increasing or decreasing MAP were defined as 0 and 100 units. MOR decreased BRS from 1.52±0.3 to 3.1±1.1µmHg and resting (R) SNA from 84±3 to 461±14. The BRS and R-SNA were unaffected by NAL-CVL (1.56±0.23µmHg and 121±26µmHg) and NAL-RVL reversed BRS (1.8±2.3µmHg) and R-SNA (112±6 µmHg) and caused a rightward shift in the SNA vs MAP curve. NAL-CVL did not alter BRS (1.8±5.0µmHg), shifted the curve back to baseline and decreased R-SNA (591±13µmHg). SNA range was dampened after MOR (712 to 524±9µmHg) and NAL-CVL (1614 to 771±20µmHg) while NAL-RVL increased the max SNA (154±16µmHg) beyond baseline. Endogenous opiates may act in the RVL to decrease BRS and basal level and range of SNA.


We have reported blood pressure (BP) mediated changes in neurotransmitter levels in extracellular fluid of the rostral ventrolateral medulla (RVLM) (Neurosci. Abst. 227.12 (87); 82.14 (88). We have now studied changes in neuronal firing in RVLM in response to BP changes. Male Sprague-Dawley rats, 300-350 g, were anesthetized with urethane and the femoral artery and vein catheterized for BP measurement and drug administration. The floor of the IVth ventricle was exposed and glass electrodes filled with 2% pontamine blue were lowered into the RVLM. Once a neuron was found and baseline firing frequency measured, the BP was altered by either phenylephrine or nitroprusside infusions. Other rats were given clonidine (alpha 2-agonist) or yohimbine (alpha 2-antagonist). RVLM neurons located 3.72 to 3.8 mm from the interaural line increased firing during phenylephrine-induced hypertension. Cell firing fell during nitroprusside-induced hypotension. Neurons 1.0 mm rostral to this area did not respond to changes in BP. RVLM contains neurons which change firing in direct relation to induced changes in BP.


Microinjection of L-glutamate (GLU) into the nucleus tractus solitarius (NTS) of rats decreases arterial pressure (AP) and heart rate (HR). The site at which the agent acts has largely been determined by localization of the diffusion of a vital stain injected at the same site. We have, therefore, sought to assess directly the diffusion of GLU labelled with a tracer amount of [3H]GLU by quantitative autoradiographic technique. Microinjections were made into the brain stem through glass micropipettes in anesthetized rats instrumented for recording AP and HR. The [3H]GLU (50-500 nM), the animal was killed with i.v. KCl, the animal removed and frozen, and the brain stem cut in 25 mm transverse sections that were dehydrated and fixed with formaldehyde vapor. Autoradiograms were made and the slides were stained for microscopic examination. The histologic image and autoradiograms were digitized and oriented by fiducial markings. Outlined, the orientation in 2 and 3 dimensions of the diffusion sphere to the nucleus determined, and the concentration of the agent in the diffusion area of the injection assessed. (Support: VA Merit Review, HL32205, HL14388, and HS24612.)

Immunochemical evidence has demonstrated the presence of vasopressin (AVP) in several different parts of the brain. The area postrema (AP) is a unique position to be stimulated by both central and peripheral influences on cardiovascular function. Previous cardiovascular microinjection studies of the AP have not been studied. In the present study, we have examined the hemodynamic effects of microinjected doses of AVP into the AP in normotensive rats. Male Sprague-Dawley rats were anesthetized with urethane and changes in blood pressure (BP), heart rate (HR), and body temperature (BT) were recorded through a catheter placed in the femoral artery. After limited occipital craniectomy, the AP was located by stereotaxic coordinates and the microinjection was made into the AP. Different doses of AVP (0.125 to 20 ng) were microinjected into the AP in volumes not exceeding 60 nl. Low doses of AVP showed a slight pressor effect (10±5 mm Hg/2±5 b/min for HR for 0.125 ng; n=3; intermediate dose showed biphasic (pressor-depressor) effects, and high doses showed main pressor effect (12±6 mm Hg/9±6 b/min for HR for 20 ng). Microinjections of equivalent volumes of normal saline showed no response.

The results indicate that microinjection of AVP into the AP is depressor at lower doses, but pressor at the highest doses. These observations are consistent with the view that the AP is involved in the modulation of vasopressin effects on cardiovascular regulation.


Injection of NPY or CARB into the PHN of conscious Sprague-Dawley rats results in an increased mean arterial pressure (MAP) in volumes not exceeding 60 nl. The purpose of the present study was to determine the effect of administration of NPY or CARB into the PHN on various cardiac parameters. Rats were instrumented for arterial pressure and cardiac output measurement. A cannula was targeted toward the PHN for injection of either NPY (1, 0.1, 0.01 ng) or CARB (1 mg). The increase in MAP evoked by CARB was paralleled by an increase in total peripheral resistance (TPR). Cardiac output (CO) was unchanged following CARB due to a decreased heart rate (HR) and an increased stroke volume (SV). The increase in MAP evoked by NPY was accompanied by an increase in TPR which persisted despite a return of blood pressure toward baseline levels. NPY decreased CO while increasing HR thereby causing a decreased SV which, like TPR, persisted. The changes in MAP and CO, TPR and SV, and SV and HR evoked by CARB were highly correlated, while the changes in TPR and CO, TPR and SV, and CO and SV evoked by NPY were highly correlated.

These results provide further evidence that NPY and CARB differentially affect the cardiovascular system following injection into the PHN. (Supported by HL26319, HL33292, NS07254, HL38299 and an ARRA Affiliate Fellowship.)


In rats stimulation of renal mechanoceptors by increased intraluminal pressure (IUP) produces afferent renal nerve activity (ARNA) decreases the contralateral renal nerve activity (RNNA) and increases contralateral urinary sodium excretion; a contralateral renal reflex response. Chronic treatment with capsaicin, 950 mg/kg/week, abolished the renal reflex, suggesting that capsaicin-sensitive neurons are involved. In the kidney, substance P (SP) immunoreactive nerve fibers are located in the renal pelvic wall. Therefore, we compared the ARNA responses to capsicain injection to those of renal pelvic and renal interstitial stimulation. Capsaicin injected into renal pelvis, 0.5, 5, 50, and 500 ng, increased ARNA 30±10%, 30±10%, 40±10%, and 100±10%, respectively. Capsaicin injected into renal interstitium, 50, 500, 5,000, and 50,000 ng, increased ARNA 50±10%, 20±10%, 5±10%, and 30±10%, respectively. These data suggest that the sensory neurons involved in renal reflexes are located in the renal pelvic wall and activated by capsaicin and SP.

INCREASED HYPOTHALAMIC AND MEDULLARY NOREPINEPHRINE RELEASE IN RESPONSE TO HEMORRHAGE IN THE CONSCIOUS RAT. L.M. Van, Kugler* and R.J. Falk, Dept. ofPhysiol., Univ. of Tenn., Memphis, TN 38163.

The response of extracellular norepinephrine (NE) levels in the dorsal medullary, paraventricular/hypothalamic area (P/A) to maneuvers causing sympathetic activation were examined in conscious rats. DMNE and P/A were measured by in vivo microdialysis, before and during hemorrhage (HEM, to a constant arterial pressure of 50 mm Hg), after reinfusion of HEM blood, or before and after i.v. hypertonic saline (HTS, 1.5 M NaCl at 10 ul/100/min).

Since lesions of the anteroventral third ventricle (AV3V) affect brain norepinephrine (NE) levels, a role for AV3V was examined in rats with prior AV3V lesions (AV3V-X) or control surgery (CONT). DMNE and P/A were increased during HEM and returned to baseline after reinfusion of blood in both AV3V-X and CONT rats, but P/A responses to HEM in AV3V-X rats were greater than those in CONT rats. HEM and DMNE responses were similar in CONT and AV3V-X rats. HTS did not change DMNE or P/A in AV3V-X or CONT rats, despite increases in plasma norepinephrine >35 mg/mL. We conclude that 1) DMNE and P/A increase in response to HEM, but not to HTS, and 2) the AV3V region appears to inhibit P/A, but not DMNE responses to HEM (Supported by USPHS grant HL 25877 and NASA 19L0770).
CARDIORESPIRATORY RESPONSES TO ELECTRICAL STIMULATION OF THE DORSOMEDIAL HYPOTHALAMUS OF THE RABBIT. C.G. Markgraf.

CARDIORESPIRATORY RESPONSES TO ELECTRICAL STIMULATION OF R.W. Winters*. P.M. McCabe. Y-F. Duan*. and N. Schneiderman. Dept, of control of BP, leads to retrograde labeling of cell bodies in the midbrain periaqueductal gray (PAG) and dorsomedial hypothalamus (DMH). We have demonstrated that electrical stimulation of the PAG elicits components of the defensereaction, possibly via these monosynaptic connections.

The present study assessed the autonomic and respiratory responses elicited by electrical stimulation of the DMH in the urethane anesthetized rabbit. Electrical stimulation (10 sec train, 100 Hz, 80-200 µA) of this region produced a response profile that was characteristic of the defense reaction: significant increases in BP (+11-15 mm Hg), HR (+15-22 bpm), BF (+3.0-4.5 cc/min) and RESP (+20-30 breaths/min) were observed. Taken together with results of previous studies, these data suggest that the DMH and the PAG are involved in the mediation of the defense reaction in the rabbit. The BP component of this response may be influenced by projections from these structures to the RLVM, which in turn projects to the intermediolateral cell column of the spinal cord. Supported by NIH grants HL 07426, HL 36588 and NS 24874.

EFFECT OF SUBSTANCE P ON RABBIT CAROTID SINUS BARORECEPTORS AND CHEMORECEPTORS. O. LONG AND S.L. ROOTSTOWN, OH 44272

Substance P (SP) is abundant in the carotid sinus nerve (CSN) and has been implicated as a pre- and chemoreceptor reflex. There is disagreement about the effect of SP on the baroreflex and CSN single unit discharges. We examined the effect of SP on blood pressure and heart rate in urethane-anesthetized, spontaneously breathing rabbits with bilaterally cut cervical sympathetic nerves. SP (1ug/ml, 0.2ml total) or saline was slowly injected into the left internal carotid artery. SP decreased blood pressure from 104.5 ± 6.5 to 32.7 ± 5.1 mm Hg (P<0.001, N=4) and heart rate from 326.8 ± 12.6 to 277.1 ± 20.8 beat/min. In 8 other rabbits, the carotid sinus area (CSA) was vascularly isolated and perfused with saline in Locke's solution (1ug/ml, 0.2ml/min). Heart rate decreased from 320.2 ± 13.5 to 297.8 ± 15.5. SP increased the single unit activity of 12 of 18 baroreceptor fibers but inhibited all of 20 chemoreceptor fibers (decreased to 41% of control). The inhibition of chemoreceptor fibers may be direct or indirect due to dilution of smooth muscle. However, SP may directly activate CS baroreceptors and initiate a baroreflex.


The onset of hypotension during hemorrhage is accompanied by decreases in vascular resistance, plasma NE, and sympathetic nerve activity. Naloxone reverses these effects. Our hypothesis was that the increase in sympathetic nerve activity was specific to naloxone treatment and not due only to the increase in mean arterial pressure (MAP). Male, New Zealand white rabbits were chronically prepared with indwelling arterial and venous catheters and renal sympathetic nerve activity recording electrodes. The experimental protocol was to remove venous blood until MAP decreased to < 40 mm Hg, inject naloxone (3 mg/kg), and monitor MAP, HR, and carotid sinus baroreceptor fibers. Injection of saline did not change these variables (N=6). During hypotensive hemorrhage, MAP and HR decreased to 80±10 mmHg and 228±21 beat/min, respectively. Naloxone decreased MAP and HR to 58±10 mmHg and 172±21 beat/min, respectively. These results suggest that naloxone reversed the baroreceptor fibers. The MAP response to saline injection was significantly reduced by naloxone injection (N=3) increased MAP (after 2 min) to 80±10 mmHg and increased RSNA to 215±24% of the prehemorrhagic control. Two min after saline injection, MAP was 42±4 mmHg and RSNA was 41±8% of control. alpha-adrenergic blockade with phentolamine (N=3) reduced the pressor response to naloxone so MAP was only 48±1 mmHg 2 min after injection. RSNA increased in response to phentolamine, demonstrating that the pressor response to naloxone was not due to an increase in MAP. Rather, the sympathetic response was in large part due to the increase in MAP. Supported in part by BNS-8719372, HL31218, and HL6080.


This study was undertaken to determine whether cardiac nerve fibers and mechanoreceptors respond to chemical and mechanical stimulation of cardiac receptors are different.

In ten anesthetized dogs, a cannula tied into the ascending aorta was connected to a pressurized bottle of saline or alcohol solution. The cephalic circulation was perfused at constant pressure and a hind limb and the remainder of the circulation were perfused at a constant pressure. When the blood pressure flows so that changes in perfusion pressures denoted vascular resistance responses.

A step increase in aortic root and ventricular sympathetic pressures resulted in consistent vasodilatation, but no consistent change in heart rate. Injection into the aortic root of veratridine (10-25 µg) and capsaicin (50-100 µg) resulted in similar vascular responses but also consistently resulted in bradycardia. Following repeated injections or infusion of veratridine, the responses to further injections of veratridine or capsaicin were largely abolished. These changes in ventricular pressure were not significantly changed.

We conclude that responses to chemical and mechanical stimulation of cardiac receptors are different. We think that chemical and mechanical stimulation may play a significant role in the development of vascular resistance responses.


Cardiac arrhythmia can be evoked by stimulation of reflexes and central nervous system activity, as well as by pathophysiologic mechanisms within the myocardium. Ventricular fibrillation, an arrhythmia commonly associated with sudden death, has been linked to sympathetic cardiac activity and particularly to lateralized (left stellate ganglion) hyperactivity. However, a recent study of subjects with severe congestive heart failure (CHF) has demonstrated a high rate of sudden death associated with bradyarrhythmias, suggesting a vagally mediated inhibition process. To assess the relative roles of parasympathetic and sympathetic innervation in such lethal arrhythmias, we have begun prospectively recording high-resolution EKGs, respiration, and sleep-state variables (EEG, EOG, etc.) in subjects with severe CHF in the UCLA Heart Failure program. Initial analysis of heart rate variability data confirms reduced sinus arrhythmia (SA) previously reported in CHF. Two basic patterns have emerged: 1) low responses to low-frequency stimulation; overall SA with the virtual absence of low-frequency (sympathetic) components as well. The initial low-responsiveness suggests, in the absence of functional desensitization, the strong inhibition of vagal cardiac activity in severe CHF. It is possible that potentially lethal bradycardia may be allowed by the return of previously inhibited vagal activity during hemodynamic fluctuations with treatment for CHF.
We tested this hypothesis by making bilateral lesions of the habenula. A lethal dose of amphetamine (75mg/kg) was administered intraperitoneally to rats. Ninety-two percent of control animals showed no changes in glucose utilization in a mean time of 13.3 ± 0.5 min., and 100% of animals died in a mean time of 55.0 ± 9.0 min. Animals were pretreated with haloperidol in a dose range 1.0-20mg/kg showed statistically significant efficacy in reducing death (PCO.05). Propranolol afforded significant protection against amphetamine-induced death by doses of 20 and 30mg/kg (PCO.05). A combination of haloperidol (1.0mg/kg) and propranolol (10mg/kg) completely prevented amphetamine-induced death in lesioned rats that were not previously lesioned with 6-OHDA. Animals pretreated with diazepam (1.0 to 10mg/kg), yohimbine (2.5 and 10mg/kg), or prazocin (5 and 10mg/kg) had no significant difference in death incidence compared to controls. Diazepam (5 and 10mg/kg) was the only agent to reduce the incidence of seizures.

During withdrawal from amphetamine (AMPWD), both lesioned and sham rats showed decreases in square crossing as compared to controls. No significant difference in death incidence was observed. The greatest differences between lesioned and sham rats were observed around 3 inch centers. Thus, the habenula is involved with AMP-induced behaviors, but its role is a complex one.

The effects of habenula lesions on amphetamine-induced behaviors and glucose utilization in rats. R.L. Vuong+, L. Jackson+, S. Caldecott-Hazard (SPON), J. Tyszack, LBS, UCLA, and Dept. Psychiatry, VAMC, Sepulveda, CA 91343.

Previous studies have shown that the administration of, or withdrawal from, amphetamine (AMP) alters glucose utilization in the habenula. In addition, lesion of the habenula with 6-OHDA causes decreases in glucose utilization in several brain areas, suggesting that the habenula serves a functional role for this brain area in AMP-induced behaviors. We tested this hypothesis by making bilateral lesions of the habenula in rats (F1(1,20))=5.90, p<.05). The lesioned rats were then tested in the Behavioral Pattern Monitor, a 1 x 2 ft box equipped with 3 cm diameter holes in the side walls. The rats were first tested at 6 days following their first-injection (the "short-term" group), while the rest were tested at 14 days after their first-injection (the "long-term" group). The injection schedule for the long-term group began first so that all animals could be tested at the same time. All animals were tested for 1 hr in the Behavioral Pattern Monitor, a 1 X 2 ft box equipped with photocell beams arranged in a X-Y coordinate system around 3 inch centers. Overall activity during the hour-long session was decreased in the short-term MDMA rats (F1(1,20)=15.90, p<.001). Crossovers between the two groups showed that this decrease was due to the acute effects of AMP (F1(1,20)=4.50, p<.05). Entries into the corner regions of the BPM were reduced (F1(1,20)=4.31, p<.05), and a trend toward decreased entries into the corners was observed (F1(1,20)=3.57, p<.07). For long-term MDMA rats, no significant effects on these behavioral measures were observed. Thus, short-term SRI involvement in behavioral effects, with compensatory adaptation long-term, is suggested.
469.7

DIFFERENTIAL REACTIVITY TO ENVIRONMENTAL AND PHARMACOLOGICAL CHALLENGES PREDICTS INDIVIDUAL VULNERABILITY TO AMPHETAMINE SELF-ADMINISTRATION. P.V. Piazza, J.M. Deminière*, M. Le Moal, H. Simon*. (SPON: C.M. Thienot-Blais)

The objective of the present work was to analyze the following question: are there inherited or acquired predisposing factors indicative of the future susceptibility to amphetamine self-administration (SA) in rats? In view of the strong relationships between stress and the activity of dopaminergic neurons on one hand and between dopaminergic neurons and SA behavior, on the other, we studied two factors: the behavioral reactivity to stress and the alteration of dopaminergic neuronal activity by repeated stimulation with amphetamine injections (sensation procedures).

We showed that Sprague-Dawley rats originating from the same breeding center exhibited a great variability in their locomotor response in a novel environment (scores from 200 to 1200 in 2 hours). This individual difference in the reactivity to a mild stress was positively correlated (r=0.47, p<0.01) to the individual sensitivity of DA neurons measured by the locomotor response to an i.p. injection of amphetamine (doses from low to high). In fact, we showed that the administration of a low dose of amphetamine (10mg/kg) in rats with a high baseline level of DA and a low percentage of DA variance in a novel environment produced a significant increase in mean locomotor activity. However, in the group with a low baseline level of DA and a high percentage of DA variance in a novel environment, the amphetamine treatment produced no increase in mean locomotor activity. Thus, the repeated activation of DA neurons provokes the development of SA behavior in those animals previously hypoactive to stress and to amphetamine.

These results may provide a psychological basis for addiction liability observed in humans.

469.9


Vulnerability to drug intake shows individual differences between the rats of the same strain. In this work, we were interested in characterizing these differences both at the behavioral and biological level. The activity of the hypothalamic-hypophyseal-cortico-adrenal axis, as measured by the plasmatic concentrations of corticosterone, was studied in correlation with the sensitivity to stress and with the susceptibility to develop an amphetamine self-administration (SA) behavior. The results obtained were the following: 1) the rats with a low basal level of corticosterone were low-responders in a novel environment and did not acquire the SA behavior. On the contrary, rats with a high basal level of corticosterone showed a high locomotor response to novelty and rapidly acquired SA, 2) while the level of corticosterone returned to basal level 2 hours following exposure to novelty in low-responder rats, it attained more than 300% of the initial concentration in high responders.

3) Exposure to novelty immediately before the SA test increased the drug intake in rats. The characterization of typologies may be useful in identifying the individual at risk for the development of drug abuse.

469.10


Rats were trained to discriminate 1.0 mg/kg amphetamine (ip) from saline, and then tested for generalization to a range of amphetamine doses (0.0, 0.25, 0.50 & 1.5 mg/kg) injected either alone, or after pretreatments with nicotine, midazolam, morphine or ethanol. Nicotine (2.0 & 0.4 mg/kg, sc) both potentiated partially and augmented the stimulus properties of amphetamine, so that the amphetamine stimulus generalization function was relative to the curve obtained without nicotine. Midazolam (0.10 & 0.20 mg/kg, sc) and morphine (2.0 mg/kg, sc) attenuated the stimulus properties of amphetamine causing the generalization functions to be lowered relative to control curves. Ethanol (0.5 & 1.0 g/kg, ip) enhanced the cueing effects of amphetamine, but did not produce generalization when injected alone. These results demonstrate that the stimulus properties of amphetamine may be affected by psychoactive drugs from a variety of pharmacological classes.

469.11

SEQUELAES OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN HUMANS: PREDATORY FINDINGS. L.H. Price, M.D., C.A. Ricart*, M.D., Ph.D., J.H. Krystal, M.D., G.R. Henningfield, M.D. Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT (06520).

3,4-methylenedioxymethamphetamine (MDMA= "Ecstasy"), a selective serotonin (5-HT) neurotransmitter in laboratory animals, has achieved notoriety as a new "hallucinogenic" and "stimulant" drug in psychiatric clinics. The potential for MDMA to produce long-lasting, psychotherapeutic potential. We studied its effects on 5-HT function by comparing responses to the 5-HT precursor L-tryptophan (L-TRP) in MDMA users and healthy controls, and comparing those effects in two different strains. In this work, we were interested in characterizing these differences both at the behavioral and biological level. The activity of the hypothalamic-hypophyseal-cortico-adrenal axis, as measured by the plasmatic concentrations of corticosterone, was studied in correlation with the sensitivity to stress and with the susceptibility to develop an amphetamine self-administration (SA) behavior. The results obtained were the following: 1) the rats with a low basal level of corticosterone were low-responders in a novel environment and did not acquire the SA behavior. On the contrary, rats with a high basal level of corticosterone showed a high locomotor response to novelty and rapidly acquired SA, 2) while the level of corticosterone returned to basal level 2 hours following exposure to novelty in low-responder rats, it attained more than 300% of the initial concentration in high responders.

3) Exposure to novelty immediately before the SA test increased the drug intake in rats. The characterization of typologies may be useful in identifying the individual at risk for the development of drug abuse.

469.12

THE COMPARISON OF THE BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF 5,7-DHT, MDMA AND 4,6-DIFLUOROMETHAMPHETAMINE. W. Nate, T. Gribbestad, and S. Lorenz (SPON: R. Schacht). Department of Pharmacology (Body, 159), Loyola University Medical Center, Maywood, IL 60153.

Two experiments are in progress. In the first, separate groups (n = 1-12) of male Sprague-Dawley rats were treated with different doses of 5,7-dihydroxytryptamine (5,7-DHT) 0.25, 0.50 or 1.0 mg/kg i.c. 10-15 min following sodium bicarbonate and desipramine, 15 mg/kg, i.p., 3,4-methylenedioxymethamphetamine (MDMA) 0.5 or 40 mg/kg, sc.; b.i.d. 4 x 4), 4,6-difluoromethamphetamine (5,7-DHT 0.25, 0.50 or 1.0 mg/kg, sc.; b.i.d. 4 x 4). Exploration of a novel open field (12 min), morphine analgesia (5.0 mg/kg, s.c.), and swimming ability (41 min) were examined in the animals 3 days immediately preceding sacrifice either one or two weeks post-treatment. In the second experiment, separate groups of rats were treated with either saline (1.0 mg/kg, s.c.; b.i.d. 4 x 4), MDMA (40 mg/kg, sc.; b.i.d. 4 x 4) or FEN (12 mg/kg, sc.; b.i.d. 4 x 4). The animals were food deprived, maintained at 80-90% their pre-deprivation body weights, and trained in a 4-arm radial maze for food reinforcement.

The neurochemical analyses completed to date show that all treatments reduce hippocampal, neostriatal, nucleus accumbens and hypothalamic 5-HT levels two weeks post-administration, but that 5,7-DHT is the most effective. The 5,7-DHT treated rats tended to exhibit increased swimming ability, and the highest dose (200 mg) may potentiate morphine analgesia eight weeks post-injection. Otherwise the 5,7-DHT rats appeared normal.

MDMA and FEN treatments failed to affect the behavior.

These and previous data suggest that repeated high doses of MDMA and FEN do not lead to dysfunctions in exploratory behavior, motor coordination or stamina, the acquisition of a one or two-way conditioned avoidance response, or spatial memory. (Supported by NIDA Contract 1737-74111L)

N-methyl-D-aspartate (NMDA) receptors have recently been linked to the decrease in tryptophan hydroxylase (TPH) activity induced by multiple administrations of methamphetamine (METH) (Johnson et al., 1989). Because NMDA receptors are linked to a calcium channel, in this study we examined the ability of the calcium channel antagonist, fluranizine (FLU), to alter the changes in TPH activity induced by METH or 3,4-methylenedioxy-methamphetamine (MDMA). Male Sprague-Dawley rats (180-240 g) received 4 doses of either METH (15 mg/kg, s.c.) MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-hr intervals. The animals were killed 18 hr after the last drug administration. METH and MDMA administration induced a decrease in TPH activity to 64% and 38% of control, respectively. Coadministration of FLU with METH further reduced TPH activity to 40% of control while coadministration of FLU with MDMA provided significant protection from the MDMA-induced decrease in TPH activity as enzyme activity was lowered to only 78% of control. These results suggest that calcium may participate in the METH-induced decline in central TPH activity, but the mechanism by which METH decreases TPH activity may differ from MDMA.

(Supported by grants DA 00869, DA 04222 and MH 44454)

4-METHYLAMINOREX possesses two chiral centers and exists as cis and trans geometric isomers. In this study, we evaluated, in tests of stimulus generalization, the effects of its (+)- and (-)-isomers all produced (+)amphetamine-like responding; ED50 values are (+)cis: 1.5 mg/kg, (+)trans: 1.5 mg/kg, and (-)trans 4-MAX (15-mg psii) in male S-D rats trained to discriminate 1.0 mg/kg of (+)amphetamine sulfate (i.p.) from saline in a standard 2-lever operant procedure under a VI 15-sec schedule of reinforcement for food reward. The cis racemate and its (-)-isomer all produced (+)amphetamine-like stimulus generalization, whereas the (+)-isomer failed to alter the decline in TPH induced by MDMA. Administration of phencyclidine (20 mg/kg) mimicked the effect of 4-MAX on the METH-induced decline in serotonin (5-HT) without altering the effect of MDMA on 5-HT levels. The results suggest that NMDA receptors may be involved in the METH-induced changes in the central 5-HT system, while their role in the MDMA-induced changes remains uncertain. (Supported by grants DA 00869, DA 04222 and MH 44454)

Extracted recordings were used to examine the responses of 78 neurons in the rat nucleus submedius (SM). Responses were obtained from 13/18 cells activated only by noxious stimuli of swift onset and rapid termination. In contrast, responses of 3 cells were delayed both in onset and termination, and in 2 neurons the response was evoked activity. The responses of 12 neurons were of rapid onset and terminated after a brief latency. The responses of 7/12 neurons activated only by innocuous stimuli were of swift onset and rapid termination, while that of 3/12 were delayed in both onset and termination. Two of the 12 innocuous-only cells became unresponsive to repeated stimulation, and could be re-activated only after a period during which no stimuli were applied. The remaining 39 SM neurons were not activated by mechanical cutaneous stimulation. Electrical stimulation of the ventral spinal cord was used to examine projections of 1/39 characterized SM neurons. Ten cells were activated systematically, while two neurons were inhibited.

These results suggest that the nociceptive function of SM neurons in nociception and additionally suggest that their role is not limited to nociceptive information signaling but encompasses a wider range of cutaneous sensations. (Supported by NS26650).


Previous studies (Craig & Burton J. Neurophysiol. 45, 443, 1981, Craig et al. Proc. Natl. Acad. Sci. USA 75(8), 4500, 1978) have shown that neurons in rat SM respond to nociceptive inputs, but the anatomical connections of SM neurons have not been characterized. In the present study, injections of wheat-germ agglutinin horseradish peroxidase were made in the rat SM or VLO. Following a survival period of 4-5 days, the animals were perfused and tissue sections were processed with the TMB reaction. Following injection into VLO, dense retrograde and anterograde labeling was observed in SM. Some retrogradely labeled cells were also observed ipsilaterally in medio-dorsal thalamic nucleus. No labeled cells were observed in the contralateral SM or VLO. Following SM injections, many contralaterally and some ipsilaterally labeled neurons were observed in the contralateral VLO and prefrontal cortex and the V nucleus and SM. Despite many similarities between these projections, the difference in the pattern of projections from SM to prefrontal cortex and the V nucleus and SM may reflect differences in the rostral-caudal aspects of the SM.
The possible role of morphological type and frequency of CGRP contacts: a study on CGRP in the rat spinal cord. Immunoreactive fibers from the spinal cord ascend from the ventral lateral pons and coursed with the ventral spino- cerebellar tract prior to entering the parabrachial nucleus. PHA-L immunoreactive fibers of fine caliber with en passant and terminal boutons were found primarily in the rostral part of the lateral parabrachial nucleus contralateral to the injection site. Single fibers with several branches and numerous boutons were observed to cover large portions of the lateral parabrachial nucleus dorsal to the lateral pole of the superior cerebellar peduncle. Fibers with a lower density of boutons were observed rostrally in the medial parabrachial nucleus, nucleus Koller-Fuse, and within the superior cerebellar peduncle. Fibers with a lower density of boutons were observed rostrally in the nucleus cuneiformis. Projections to Koller-Fuse were heaviest when the injection site was not limited to lamina I, supported by grants NS34043 and NS31443.

**ANANTOGENIC EFFECTS OF LESIONS IN ANTEROLATERAL COLUMNS (ALC) AND DORSOLATERAL FUNICULUS (DLF) ON REACTIONS TO ACUTE AND CHRONIC NOISY STIMULI IN RATS**

The possible role of lesions in anterolateral columns (ALC) and dorsolateral funiculus (DLF) on reactions to acute and chronic noisome stimuli in rats has been explored. Animals were injected with retrograde tracers to identify spinal descending inhibitory mechanisms. The results suggest that lesions in ALC and DLF may affect descending inhibitory mechanisms. The study was supported by the National Science Foundation.

**COLLABORATION OF SPINO-MESENCEPHALIC TRACT (SMT) AXONS**

The collaboration of spino-mesencephalic tract (SMT) axons in the rat was studied using antidromic stimulation to map the SMT axons and their projections. The results showed that SMT axons have multiple projections, including to the midbrain, and that these projections are modulated by supraspinal inputs.

**EFFECTS OF VARIOUS SPINAL LESIONS ON AUTONOMIC (AT) AND SPINAL MECHANISMS**

The effects of various spinal lesions on autonomic (AT) and spinal mechanisms were studied in rats. The results showed that different types of lesions produce distinct effects on autonomic and spinal mechanisms, including changes in heart rate, blood pressure, and other autonomic functions.

**HURSDAY PM PAIN PATHWAYS: CNS**

The session focused on pain pathways in the CNS. The presentations covered various aspects of pain research, including nociceptive mechanisms, pain modulation, and the role of the spinal cord in pain processing.

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**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989**

The session focused on pain pathways in the CNS. The presentations covered various aspects of pain research, including nociceptive mechanisms, pain modulation, and the role of the spinal cord in pain processing.
ASCENDING LAMINA I AXONS IN THE CAT ARE CONCENTRATED IN THE MIDDLE OF THE LATERAL FUNICULUS. A. D. Craig, Jr. Divisions of Neurobiology and Neurology, Barrow Neurological Institute, Phoenix, AZ 85013.

The ascending axons of specifically nociceptive and thermoceptive lamina I neurons from the distal spinal tracts (ST7) in the cat, but their role in pain and temperature sensation has been challenged by reports that they ascend in the dorsolateral funiculus. Direct observations have been made following injections of the anterograde tracer PHA-L in lamina I at different spinal segments. Ascending lamina I axons are variably located throughout the contralateral white matter, but in general are concentrated in the middle of the lateral funiculus, i.e., at the level of the central canal. This finding is consistent with the location of lesions affecting thermoception in cat and, given the increase in size of the corticospinal tract in man, with several descriptions of anterolateral chordotomies. The retrograde labeling data reported earlier by others may have been complicated by the transneuronal retrograde transport of WOAMRF, its weak efficacy for labeling lamina I STT neurons, and the inherent variability in the location of ascending lamina I axons.

(Supported by NS 25616 and the Barrow Neurological Foundation.)

SPECTRAL EEG CHANGES WITH COLD PRESSOR.


Although severe pain in humans, especially chronic pain has profound effects on affective and cognitive supraliminal processes, physiologic data for changes in these processes are slight.

Spectral analysis of EEG of 4 healthy subjects was recorded during immersion of the right hand in painful cold water (0-2°C) and during immersion of the right hand in warm water (20°C) control. Averages of the spectrum from 13 artifact free epochs (five seconds each) were used to calculate an asymmetry ratio (right-left/right+left) for 6 pairs of electrodes. Repeated measures analysis of variance of these asymmetry ratios for 4 subjects revealed significant effects for electrode and electrode by pain effects in the alpha band but not in the delta or theta bands. Post-hoc comparisons revealed that only the posterior electrode pairs (P3-P4, T5-T6) showed significantly higher asymmetry ratios during cold pressor than during control stimulation. These ratios reflect relatively greater right sided alpha power. Physiologic implications will be discussed in the presentation.

JOINT AND MUSCLE A- AND C-AFFERENT FIBER CONVERGENCE IN RAT TRIGEMINAL (V) BRAINSTEM NEURONS. J. W. Hu*, Y. Sharav* and B. J. Neagle. Faculty of Dentistry, University of Toronto, Toronto, Ontario, M5G 1G6, Canada.

The present study was initiated to examine possible deep afferent inputs from temporomandibular joint (TMJ) and hypoglossal (XII) muscle afferents to the V subnucleus caudalis (medullary dorsal horn). Extracellular single neuron recordings were made from caudalis of 14 rats anesthetized with urethane-chloralose. Neurons were classified on the basis of their cutaneous mechano-receptive field properties as low-threshold mechano-receptive (LTM, n=81) and nociceptive-specific (NS, n=27). TMJ or XII electrical stimulation excited respectively 8% and 50% of the LTM neurons, 64% and 50% of the WDR neurons and 15% and 43% of the NS neurons. Latencies of responses to TMJ stimulation were longer than those to skin electrical stimulation but shorter than those to XII stimuli. 50% of the neurons had A and C fiber inputs evoked by high-intensity (2ms, 5 mA) stimulation of TMJ and XII as well as skin, whereas only 15% of NS neuron and no LTM neuron had an A as well as a C fiber TMJ or XII input. These results provide important insights into the organization of deep inputs from TMJ and masticatory muscles to central V nociceptive pathways and suggest that the demonstrated convergent mechanisms in caudalis may play a role in mechanisms of deep pain in the orofacial region. (Supported by NIH grant DE-05786).

JOINT AND MUSCLE A- AND C-AFFERENT FIBER CONVERGENCE IN RAT TRIGEMINAL (V) BRAINSTEM NEURONS. J. W. Hu*, Y. Sharav* and B. J. Neagle. Faculty of Dentistry, University of Toronto, Toronto, Ontario, M5G 1G6, Canada.

The present study was initiated to examine possible deep afferent inputs from temporomandibular joint (TMJ) and hypoglossal (XII) muscle afferents to the V subnucleus caudalis (medullary dorsal horn). Extracellular single neuron recordings were made from caudalis of 14 rats anesthetized with urethane-chloralose. Neurons were classified on the basis of their cutaneous mechano-receptive field properties as low-threshold mechano-receptive (LTM, n=81) and nociceptive-specific (NS, n=27). TMJ or XII electrical stimulation excited respectively 8% and 50% of the LTM neurons, 64% and 50% of the WDR neurons and 15% and 43% of the NS neurons. Latencies of responses to TMJ stimulation were longer than those to skin electrical stimulation but shorter than those to XII stimuli. 50% of the neurons had A and C fiber inputs evoked by high-intensity (2ms, 5 mA) stimulation of TMJ and XII as well as skin, whereas only 15% of NS neuron and no LTM neuron had an A as well as a C fiber TMJ or XII input. These results provide important insights into the organization of deep inputs from TMJ and masticatory muscles to central V nociceptive pathways and suggest that the demonstrated convergent mechanisms in caudalis may play a role in mechanisms of deep pain in the orofacial region. (Supported by NIH grant DE-05786).

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THE ULTRASTRUCTURE OF TRIGEMINAL VASCULAR CONVERGENCE
NEURONS. S. Potrebic*, A. Strassman, R.A. Hartvig*. R.
McLachlan. Pain Physiology Laboratory, Mass General
Hospital, Boston MA 02114.

Brainstem trigeminal vascular convergence (TVC) neu-
rons receive an excitatory, nociceptive input from cra-
niol blood vessels as well as facial skin or cornea.
TVC cells may mediate the pain associated with vascular
headache. In the present study, cat TVC neurons were
electrophysiologically identified and then intracel-
larly labelled with horseradish peroxidase. One popu-
lation of TVC has axons which collateralize ex-
tensively in the trigeminal complex. Representative
neurons of this class were examined with electron mi-
croscopy. The labeled TVC neurons are located in ven-
trolateral lamina 1V and V of trigeminal nucleus cau-
dalis(TVC). The cells have myelinated axons and col-
laterals that give rise to unmyelinated preterminal
processes. Within laminae IV and V of TVC, terminals
of TVC cells contain round synaptic vesicles and form
synapses on dendrites, spines, and, less frequently, on
cell somas. Based on these morphological features, we
hypothesize that at least some TVC cells which col-
lateralize in the trigeminal complex provide excitatory
input to other trigeminal neurons. These cells may play
a role in the cutaneous hyperalgesia associated with
vascular head pain.

IN VITRO CONTRACTILE PROPERTIES OF MOTOR
UNITS IN ADULT HAMSTER DIAPHRAGM. M. Fournier
and G.C. Sieck. Department of Biomedical Engineering, Univer-
sity of Southern California, Los Angeles, CA 90089.

The physiological properties of diaphragm(MU) motor units were
studied using an in vitro preparation. While perfusing the ani-
mal with oxygenated Ringer's, the right hemidiaphragm was dissected
in continuity with the phrenic nerve and its cervical ventral roots.
Thereafter, these nerve-ventral root preparation was placed in a
temperature-controlled(27°C) chamber continuously perfused with
oxygenated Ringer's. Stimulation of ventral roots C 5 to C 7 indicated
somatotopy in DIA innervation. Motor units were isolated by dissec-
tion and graded stimulation (using a suction electrode) of ventral root
filaments. Based on "sag" and a fatigue test, MU were classified into
categories, based on the maximum tension of ~120

In this study, the amplitude and half-relaxation times from 34 to 160
mg compared to an agonist stimulus. Maximum tetanic tensions of MU ranged from 96 to 3.3 g compared to an ag-
gregate maximum tension of ~120 g for the entire costal region. These
results indicate that the adult hamster DIA is comprised of MU varying
considerably in their physiological properties. Furtherm ore, the
in vitro preparation provides a valuable controlled environment in which to study
MU properties.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

DETECTION AND CORRECTION OF EMG INTERFERENCE
DURING NERVE CUFF RECORDING. E.M. Eby, C.L. Cieland,
J. Brandt*, F. Gettine and J. Maloney*. Dept. Obst. and Gynecol.,
University of Calgary, Calgary, AB, Canada T2N 4N1 and Dept.
Physiol. and Biophysics, University of Iowa, Iowa City, IA 52242

Chronic recording from nerves using cuff electrodes is common in research and has prosthesis applications. However, the small
signal can be swamp ed by the electrical activity of nearby
muscles(EMG). Current techniques for suppressing EMG, such as
tripolar recording and filtering, can be inadequate. Thus, we
developed a technique in which a second set of electrodes on the
external surface of the cuff, which record only EMG, can be used
to detect and correct for EMG contamination of the neural signal.

Detection: Tripolar cuff electrodes, with both internal and external
electrodes, were implanted on the vagus nerve in lambs. The signal recorded by the external electrodes proved a useful
indicator of when neural signals were contaminated by EMG.

Correction: If EMG recorded by the external and internal
electrodes were identical, then subtraction would eliminate EMG
from the internal signal. This hypothesis was tested using a
bipolar electrode in a saline bath and a moveable electrode dipole
as the EMG source. We found that the external and internal
signals were similar only when an additional tube was placed around the cuff, producing similar current flow across the
internal and external electrodes. The subtraction of the external
from the internal signal can attenuate interference from nearby
muscles and other external current sources.

Funded by Alberta Heritage Foundation for Med. Res. and NIH.

COMPARISON OF FIBER SUBTYPE DISTRIBUTION OF
PHARYNGEAL DILATOR MUSCLES AND DIAPHRAGM IN CAT. 
R. van Lunteren and T.E. Dick. Department of Medicine, Case
Western Reserve University, Cleveland, OH, 44106.

Differences exist between the pharyngeal dilator muscles and the
thoracic inspiratory muscles in their patterns of electrical and
mechanical activity during the respiratory cycle, both in normal
humans and animals and in subjects with sleep-related
ventilatory disorders such as obstructive sleep apnea. Little is known about the
intrinsic properties of the pharyngeal muscles, however, and how they
contribute to ventilatory control. In this study, we observed
the pharyngeal dilators to be composed of three main fiber types: small
muscles (S), medium (M) and large (L) fibers. The
majority of S fibers were found in the geniohyoid and sternohyoid
muscles, with a small proportion of M and L fibers. The
majority of M fibers were found in the geniohyoid and sternohyoid
muscles, with a small proportion of S and L fibers. The
majority of L fibers were found in the geniohyoid and sternohyoid
muscles, with a small proportion of S and M fibers. These
differences suggest that the pharyngeal dilators may play a role in the
control of upper airway patency during sleep.

INTERSEGMENTAL EXCITATION OF CERVICAL INSPIRATORY NEURONS
BY LOWER INTERCOSTAL NERVE AFFERENTS. R. Shannon, Y.M.
Hernandez* and B.G. Lindsey. Dept. Physiol. &
Biophysics, Col. Med., Univ. South Florida, Tampa, FL
33612

We previously reported (Soc. Neurosci. Abst. 14:626) that 50% of the inspiratory cells located in the cervical
spinal cord (C1-2) of anesthetized cats were excited by lower (T9-10) intercostal nerve afferents (Ib from tendon
organs) through intersegmental pathways. We conducted
experiments to determine if the lack of an excitatory response in some I-cells resulted from anesthet-
ic depression of the reflexes. Studies were performed on 4
unanesthetized mid-collilellar decerebrate cats that were
thoracotomy, paralyzed and ventilated. Extracellular
cortical neuron and phasic (A2) afferent activities were
recorded. Electrical stimulation of low threshold T9-11
internal intercostal nerve afferents, which excited
phasic activity through intersegmental pathways, elicited an excitatory response in only 20 of 38 (54%) I-
cells. All cells and the phrenic decreased activity following the excitatory stimulus.

These results do not resolve whether anesthesia was
responsible for the lack of an excitatory response in
some I-cells in anesthetized cats. The results suggest that suprapontine structures participate in the gating of
the excitatory effects of lower intercostal tendon organ
afferents on cervical I-cells. (Supported by NSF
Research and Creative Scholarship Grant)

We now report on the behavior during FV of Botzinger (BOT) E neurons, which make inhibitory connections (HFO) with phrenic neurons (3) in decerebrate paralyzed cats, bilateral PHR and all ABD discharges. HFO spectral peaks were absent in PHR; and both PHR and ABD spectra had broad bell-shaped curves. We also found that the coherence (0.7-0.9) between HFOs of opposite PHRs was new during VOM. In two cats, PHR and all ABD discharges were recorded during VOM produced by emetic drugs (amphamine, lobeline, protoveratrine) or by electrical stimulation of the afferent vagus (0.5 ms, 200-500 microamp pulses at 25/s). During control respiration, PHR activity had a late expiratory ramp pattern, but this pattern was absent during FV. We conclude that during VOM: a) the inputs to PHR motoneurons from the medullary inspiratory pattern generator (which produces HFOs) are shut off; b) both PHR and ABD motoneurons receive inputs from another (unknown) pattern generator, but these inputs are not synchronized on a short time scale. (Supported by N.I.H. grants HL-27300 and HL-20585.)

INWARD RECTIFICATION IN BULBOSPINAL NEURONS LOCATED IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS OF THE GUINEA PIG. M.S. Dekin, T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-02251

The ventral part of the nucleus tractus solitarius (vNTS) of the guinea pig comprises the dorsal respiratory group. Two classes of bulbospinal neurons have been identified in the vNTS (Dekin et al., J. Neurophysiol., 58(1):195, 1987). In this report, an inward current activated by membrane hyperpolarization is described for both classes of bulbospinal neurons. All experiments were done using an in vitro brainstem slice preparation from adult guinea pigs. When voltage clamped at a membrane potential of -60 mV in the presence of 0.5 ug/ml tetrodotoxin, all neurons responded in an ohmic manner to voltage steps down to -70 mV. For voltage steps between -90 and -40 mV, inward rectification was observed. The size of the inward current increased as the voltage step became more negative. Peak inward current was strongly voltage dependent, and the slope conductance (mS/mV) was observed and required several hundred msec to fully develop. The amplitude of the inward current increased as the voltage step became more negative. Peak inward current was strongly voltage dependent, and the slope conductance (mS/mV) was observed and required several hundred msec to fully develop. Inward rectification was observed. The size of the inward current increased as the voltage step became more negative. Peak inward current was strongly voltage dependent, and the slope conductance (mS/mV) was observed and required several hundred msec to fully develop.
471.11 EFFECT OF LESIONING THE PARABRACHIAL NUCLEI ON THE PHRENIC NERVE RESPONSE EVOKED BY NASAL MUCOSAL STIMULATION. T. M. Hinchcliff, N. G. Dignan and N. G. Chemelli. Departments of Medicine and of Physiology and Biophysics, Case Western Reserve Univ., Cleveland, OH 44106.

Stimulation of the upper airways by noxious agents evoke changes in breathing pattern which presumably help defend the airways. The neural pathway for this reflex is unknown. However, the parabrachial nuclei receive a strong projection from paraganglionic neurons and contain neurons that have phasic activity related to the breathing of the naso-pharyngeal region (referred to as the pontine respiratory group (PRG). We tested the hypothesis that the PRG is important in mediating respiratory defense reflexes by assessing the responsiveness to electrical stimulation of the nose mucosa before and after lesioning the parabrachial nuclei in newborn rats (1-3 weeks of age).

471.12 HIGH PRESSURE REDUCES SENSITIVITY TO ALTERATION IN PH IN ISOLATED MEDULLA SPIRAL-CORD OF NEWBORN RATS. A. Tarasik and Y. Grossman. Unit of Neuropharmacology, Department of Neurosciences, Ben-Gurion University, Beer-Sheva 84105, Israel.

High pressure (HP) induces neurological symptoms associated with various respiratory difficulties. We examined the effect of HP on the sensitivity of the respiratory center to alteration in pH. The medulla and spinal cord were isolated from anesthetized newborn rats, placed in a pressure chamber and superfused with 95% O2 - CO2 Ringer’s solution. Solutions with pH of 6.7-7.6 were obtained by either equilibration with modified Ringer (pH=7.2) or adjustment of the CO2 content for P(CO2) so far for P(CO2) than (N2O2). Exposure to pH 6.8 (P(CO2)) reduced by 22% the time integral of a single respiratory burst in C1, but not in C2. HP reduced by 60% the sensitivity of the system to alteration in pH by both methods. In addition, the time integral of both C1 and C2 responses became independent of P(CO2) causing a relative change in "Respiratory Drive" (time integral x frequency) between the two responses. These modifications in the chemoresponse of the respiratory center correspond to the respiratory problems encountered under HP conditions.


In order to study the generation of respiratory activities in the brainstem we used the isolated perfused brainstem of adult guinea pig. The brainstem was rapidly removed from the cranium, the basilar artery was cannulated at the pontine level and was perfused rostro-caudally with O2-CO2 saturated solution. The central respiratory drive was recorded from the ventral roots of the hypoglossal nerve since the rhythmic activities of hypoglossal and phrenic nerves are called "in vivo". Neural activities were recorded in the brainstem through a 2-barrelled electrode containing a dye to mark the recording sites. Different types of spiking and rhythmic discharges were identified: 1) repetitive single spikes or bursting activity with a firing rate of 3-10/sec. 2) trains of 5-20 spikes separated by long intervals giving a spike-train frequency of 2-75 trains/min. Some of these latter periodic discharges were recorded from a phrenic nerve and a C1 cervical root using suction electrodes.


The in vitro brain stem - spinal cord preparation of the neonatal rat is used to study mechanisms of respiratory pattern generation. The rhythms reported to date have consisted of slow single bursts (0.5-1 s duration, 0.1-0.2 Hz). We report here a different pattern, to be termed "multiple bursting", in which 3-25 consecutive bursts (0.3-1 s duration, 0.5-1 Hz) form a "group" and such groups appear periodically at 15-40 s intervals.


Neurons with firing rates modulated in phase with the respiratory and/or cardiac cycles are distributed in the midline of the brainstem. The cooperative behavior of these neurons was studied in anesthetized (Dial), paralyzed, bilaterally vagotomized, artificially ventilated cats. 73 samples of 4-9 simultaneously monitored midline neurons were recorded in the nucleus tractus solitarius and ventrally in the ambiguous and paraganglionic reticular nuclei. These results show that rhythmic function is present in the isolated brainstem which may be an useful tool to study the network responsible for respiratory generation.


Cardio-respiratory effects of TTX (15 ug/kg; single ip dose) were studied in urethane-anesthetized guinea pigs instrumented for concurrent monitoring of medullary respiratory-related units (RRUs), diaphragm EMG (DERM), ECG, EKG, blood pressure (BP), arterial O2 and CO2, end-tidal CO2, vagal traffic and core temperature. TTX consistently produced a state of progressive hypercapnia which lasted the entire course of intoxication. BP responses initially showed a hyperemic profile. This was followed by a profound, time-dependent decrease in respiratory cycle frequency; b) an elevating RR spike frequency; and c) a marked increase in inspiratory RRs' Te/Ti ratios and a modest decrease in inspiratory Ti/Te ratios which is indicative of fundamental changes in the bulbar respiratory rhythmic mechanism. Other changes included a slight but steadast decline in a) BP; b) DMG amplitude; and c) vagal traffic. The central respiratory rhythmic mechanism remained unchanged up to the point of respiratory arrest. Simultaneously recorded inspiratory and expiratory RRs showed that respiratory requirements changed from respiratory-excitatory to respiratory-suppressant.

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Experiments on adult (A) and newborn (NB) rat and adult turtle brainstem slices were undertaken to determine the sensitivity or tolerance to hypoxia (P02= 10-15 Torr) of brainstem neurons. Intracellular recordings showed that adult rat hypoglossal neurons (n=23) depolarized by 31.7 ± 9.2 mV during exposure to 5 min. of hypoxia while NB (age= 3-16d) (n=47) depolarized by about 11.5 ± 5.6 mV, NB cells could be exposed to hypoxia for more than 15 min. before they depolarized by 70mV. The hypoxic depolarization in A is most likely a postsynaptic phenomenon since TTX, Ca++, free or low Ca++, high magnesium solutions did not affect the depolarization trajectory. [K+] in the brainstem extracellular compartment (ECF) increased much more in A (±5.0mM) than in the NB (±0.5mM) during hypoxia. Adult rat brainstem neurons (n=10) tolerated complete anoxia for more than 30 min. at 23°C with little change (±3 mV) in membrane potential or firing frequency. Tolerance to anoxia in turtle neurons did not change when recordings were made at the same temperature (36°C) as that for rat recordings. We conclude that 1) the difference in the magnitude of depolarization during hypoxia between A and NB rat neurons is, at least in part, due to the difference in ECF [K+]; 2) the tolerance to O2 deprivation in turtles is much greater than that of A or NB rats and 3) the increased tolerance in turtles is not related to the lower brain temperature in these animals.


The hippocampal formation of the cat exhibits rhythmic slow electrical activity during periods of somatic activity, with the frequency and amplitude of this activity being a function of particular motor acts. Envelopes of this rhythmic activity are correlated with phasic diaphragmatic EMG bursts, with the phase relationship dependent on period length of phasic bursts. This apparent relationship of hippocampal electrical activity to respiratory patterning may be an epiphenomenon reflecting simultaneous activation of motor structures, or may suggest a role, perhaps of activation, in organizing breathing patterns. We examined the effect of single (300-400 uA) electrical pulses delivered to the dorsal hippocampus during the awake state of drug-free intact, freely moving cats. Pulses delivered at rates slightly higher than base respiratory rhythms induced a switch from expiration to inspiration. These results confirm a previous study demonstrating inspiratory switching effects of stimulation delivered to the hippocampus of anesthetized cats, and suggest a more active role for this structure in organizing respiratory patterns. Supported by NIMH 22418-12.


We previously described an amplitude modulation of hippocampal 4-8 Hz rhythmic slow activity correlated with respiratory patterning during waking and rapid eye movement sleep. This study examined slow wave electrical activity from the hippocampus during two conditions that modify respiratory patterning: quiet sleep, which extends inspiratory duration, and hypothermia, which induces tachypea. Five cats were instrumented with bipolar electrodes placed in the thalamus, hippocampus and anterior ventral thal nu; EMG and EEG leads were placed in the diaphragm and nuchal musculature. Following recovery, each cat was allowed to sleep undisturbed in a quiet chamber and was administered cocaine to induce hypothermia. Temperature was measured with a rectal probe or with implanted brain or nuch muscle probes. Electrical activity from the hippocampus was cross-correlated with integrated diaphragmatic activity. Hypothermic episodes (core temperature about 39°C) were associated with very rapid respiratory rates and with enhanced involvement of upper airway muscles, including the genioglossus and facial musculature. In both quiet sleep and hypothermic conditions, diaphragmatic activity was correlated with aspects of slow wave activity: both inspiratory and expiratory components of the respiratory cycle were reflected in hippocampal electrical activity during quiet sleep. We conclude that respiratory patterning is reflected in electrical activity of rostral brain areas and that different aspects of hippocampal electrical activity are related to alterations in respiratory pattern. Supported by NIMH 22418-12.


Mecamylamine (Mec), antagonizes the action of nicotine and other nicotinic agonists both at autonomic ganglia and in brain; but it does not appear to act either competitively or allosterically at nicotinic receptors, using 3H-nicotine as the ligand. With the use of 3H-(N-methyl)-mecamylamine a study was undertaken to characterize the site of Mec's action using synaptosomes. Specific 3H-Mec binding was maximal in 10-20 sec and declined to equilibrium in 1 min. A Scatchard yielded a Kd value of 2 nM and 10% of 29 fmoles of protein; and the Kd determined from the association and dissociation rate constants was 0.4 nM. A good correlation was noted between the Kd values of a number of Mec analogues and their ability to block the peripheral and central actions of nicotine. The apparent Kd values were 0.8 nM for the 3-dimethyl, 4 nM for the desmethyl, 5 nM for the 3-ethyl analogues of Mec. Although Mec did not compete with 3H-nicotine binding to synaptosomes or membranes, nicotine and a number of its analogues competed well with 3H-Mec for binding with Kd values 20 nM but their affinities did not correlate with the psychotomimetic potency of the analogues. The findings are consistent with the hypothesis that at least Mec exerts nicotinic blockade at discrete (nicotinic) sites, and 2) nicotine itself may be exerting its pharmacologic effects by acting both at nicotinic receptors and the ionic sites.

ACETYLCHELORINE IV
neurons or are altered in age-related disorders such as Alzheimer’s disease. The processes which impart sodium dependency of the lipoxygenase pathway are chosen from those which co-exist in forebrain cholinergic preparations yielding binding consistent with a single population of presence or absence of atropine (10 uM) and incubated to equilibrium for 1 hour at 37°C. Saturation studies in control and in-vivo exposure. (Supported by the Frank H. Gower Memorial Fund, Alzheimer’s Disease and Related Disorders Assoc; grant)

472.4


The effect on cholinergic function of a novel imidazoline derivative, pyridino [1,2-a] imidazo [5,4-b] indole (IMID) was evaluated in vitro with nerve preparations of guinea pig ileum, frog sciatic nerve-sartorius muscle, and denervated rat soleus muscle. The effect on cholinesterase activity was evaluated in rat blood and histological studies of brain and spinal cord. The compound was applied in vivo by intracerebroventricular injection to the central nervous system. The imidazoline derivative was previously shown to be selective for the muscarinic M2 receptor in the procedure described previously (1, Med. Chem. 1995: 1-10).

IMID alone at 10-11 to 10-5 M concentrations did not increase developed tones in isolated ileal tissue. However, 10-9 M acetylcholine (ACh) evoked developed tension ranged from +20% to +60%. Responses were blocked by 10-8 M atropine. Inotropic (direct nerve) stimulated sartorius muscle twitches demonstrated a half-maximum effect at 119% of control with 5 x 10-6 M IMID. No effect was noted on twitches evoked by direct stimulation. In denervated rat soleus muscle, up to a 38% depression in tension was demonstrated with IMID plus ACh versus ACh alone. In the rat cortex, the responses to characteristic frequency tones were augmented (40-122%) with IMID plus ACh versus ACh alone. The compound had no effect on cholinesterase activity.

The results suggest a potentiation of cholinergic function mediated through the post-synaptic ACh receptor complex; however, an effect on acetylcholine release cannot be ruled out. [Supported in part by: NIH-563800307-17, NSF-88016837, and VAMC RAC program.]

472.3


Pilocarpine is well-established as a muscarinic agonist, displaying both M1 and M2 (and possibly M3) receptor activities. The present study includes lowering of body temperature and densities at high concentrations. Central M1 effects of pilocarpine are less well established. Binding studies using [3H]QNB in cortical sections of rat brain revealed that pilocarpine is a selective muscarinic agonist with having the following rank order of subtype selectivity: M1[(superior colliculus)M1[(nmbdithalamus)M1[(d enary gastric mucosa)M1[(submaxillary gland], with the ratio M1/M1= 106. Pilocarpine was non-specifically in vitro for its ability to stimulate PI turnover in the hippocampus (M1 response) where it displayed 35% of the on the presence or absence of atropine (10 µM) and incubated to equilibrium for 1 hour at 37°C. Saturation studies in control preparations yielded binding consistent with a single population of sites having Kd = 61 ± 6 µM and Bmax = 97.7 ± 5.1 fmol/mg tissue (mean ± SEM, n = 14). The neuropeptides VIP, CCK, ST and NT did not significantly affect Kd (89, 71, 99 and 135% of control, respectively) or Bmax (95, 91, 126 and 104% of control). Further studies in tissue from senescent rats will define possible age-related changes of this hypothesis. Very low binding is observable.

Studies of the binding properties of M1 muscarine receptors have been more difficult than similar studies of M2 receptors, because of the lack of a selective M1 agonistic compound. The different affinity of muscarine receptors for agonists and antagonists has made binding studies using the selective M1 antagonist 3-pirenzepine difficult to interpret. 3-Hydroxytryptamine (3-HO) is believed to label both M1- and M2 receptors but so far it has not been possible to show regional distributions of 3-hydroxytryptamine in accordance with this hypothesis. Very low binding is observed in areas rich in M1 receptors i.e., hippocampus whereas high levels are found in areas rich in M2 receptors (mean nH=0.98) of high affinity (mean Kŋ=5.9 M1 sites).

Nevertheless, inhibition of 4-oxo binding by pyriperazine gives biaxial displacement curves showing area dependent percentages of M1- and M2 sites. The affinity for central M1- and M2 receptors of selected standard compounds has been measured by blockade of 4-oxo binding to rat brain sections in dentate gyrus and sci.
472.9


[3H]hemicholinium-3 ([3H]HCh-3), a potent inhibitor of sodium-dependent high choline uptake (HCUP), is only slightly inhibited by prior formation of vesicles ghosts, and is strongly inhibited by vesicles, cold, low [3H] and 4-chloromercuribenzenesulfonate (MPS). Passive uptake is slightly inhibited by ATP and cold, nearly unaffected by 2-deoxyglucose, and barely affected by low [3H], MPS and other sulfhydryl modifiers and prior formation of vesicles ghosts. The ton dependent of passive and active acetylcholine transport is also different. It is concluded that passive acetylcholine is not mediated by the acetylcholine transporter but rather by a channel.

472.10

COMPARATIVE STUDY OF PASSIVE AND ACTIVE ACETYLCHOLINE UPTAKE BY SYMPATRIC VESICLES. Kleinberger and S. A. Parsons. Department of Chemistry, University of California at Santa Barbara, CA 93101.

A comparative study was made on passive and active acetylcholine uptake of synaptic vesicles isolated from the electric organ of Torpedo californica. Active transport mediated by the acetylcholine transporter is the rate limiting step in acetylcholine synthesis, binds to the carrier of SDHACU, and has high affinity for sodium concentration with high specificity. In the present study, we examined the [3H]HCh-3 binding sites that were specific for [3H]HCh-3 in bovine membranes exhibited sodium-dependency and was inhibited by HCh-3 and choline with an IC50 of 5.9 nM and 69 µM, respectively. The [3H]HCh-3 binding site was solubilized by 0.2% deoxycholate and 0.025% NaCl. The addition of 5% percent glycerol improved stability, and the binding activity remained constant up to one month when kept at 40°C. The solubilized carrier was applied to a DEAE-Sepharose column and the detergent was exchanged to 0.01% Tween 80. Protein was eluted by a linear gradient of NaCl, and the peak of binding activity was found in the fractions containing approximately 400 mM NaCl. The resin was then washed extensively and binding activity was eluted with a wash solution containing buffer. The basis of the amount of protein loaded on the affinity column and the amount recovered in the eluant was associated with [3H]HCh-3 binding; this step achieved a several thousand-fold purification.

472.11


Neurosurgery and Anatomy & Cell Biology, UCLA Neurosciences, California 90024.

We examined the effects of aminopyridines (4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP)) on acetylcholine (ACh) release from rat striatal slices superfused with a choline (Ch) containing or Ch free medium, at rest and during electrical stimulation. In Ch free medium, 4-AP (10-100 µM) or 3,4-DAP (1-10 µM) increased basal ACh release, while lowering the net efflux of Ch; thus, the ratio of ACh to Ch release was increased. Tissue ACh, Ch, and membrane phospholipid levels (including phosphatidylcholine (PC)) were not affected by aminopyridines. In a Ch (404 µM) containing medium, aminopyridines further potentiated Ch-induced ACh release. Electrical stimulation of striatal slices increased ACh release without altering Ch efflux and depleting tissue Ch-ACH stores, but depleting membranes of PC and other major phospholipids. Superfusion of the striatal slices with aminopyridines during stimulation enhanced ACh release: diminished Ch efflux; and protected the slices from stimulation-induced phospholipid depletion. Calcium dependent activation of high affinity choline uptake may underlie the observed effects of the aminopyridines. (Supported in part by grant MH-28783.)

472.12


Scanning tunneling microscopy (STM) can image atomic surfaces of metals and semiconductors. Biological STM applications are somewhat limited by poor conductivity, adsorbate layers, elasticity and poor stability of biomolecules. For direct STM observation of microtubules (MT) isolated from pig brain by standard techniques of differential ultracentrifugation, we determined optimal preparation conditions: fixation with 0.1% glutaraldehyde and solution in 0.8 M glycerol reassembly buffer (MES, BTPA, GTP, MgCl2). Both freeze dried and hydrated MT prepared in this way were reproducibly imaged in air at room temperature on graphite with a Nanoscope I STM (Digital Instruments, 135 Nogal Drive, Santa Barbara, CA 93110). The presence of MT was verified by electron microscopy. STM images of microtubules 25 nm in width, consisting of 5 to 7 longitudinal filaments of about 4 nm width: top views of MT which show about half of their 13 component protofilaments. Although MT appeared semilatticed, the known helical twist of protofilaments was clearly evident. Top view shaded scans revealed 46 nm individual tubulin subunits within protofilaments. STM and related techniques (atomic force microscopy, scanning near field optical microscopy, scanning ion microscopy) offer unique opportunities for the study of neuro­ molecular structures.

472.13


Modifications of an existing HPLC acetylcholine (ACH) assay were made to provide the time resolutions needed to detect changes in the kinetics of ACh release during behavior. WGA-HRP was administered to rats and the microdialysis probe that had been previously used for collection, allowing the anatomical localization of neurons of origin. Directly isolated periventricular onset neurons were localized in various areas of the basal forebrain known to contain ACh cells. For direct SIM observation of microtubules (MT) isolated from pig brain by standard techniques of differential ultracentrifugation, we determined optimal preparation conditions: fixation with 0.1% glutaraldehyde and solution in 0.8 M glycerol reassembly buffer (MES, BTPA, GTP, MgCl2). Both freeze dried and hydrated MT prepared in this way were reproducibly imaged in air at room temperature on graphite with a Nanoscope I STM (Digital Instruments, 135 Nogal Drive, Santa Barbara, CA 93110). The presence of MT was verified by electron microscopy. STM images of microtubules 25 nm in width, consisting of 5 to 7 longitudinal filaments of about 4 nm width: top views of MT which show about half of their 13 component protofilaments. Although MT appeared semilatticed, the known helical twist of protofilaments was clearly evident. Top view shaded scans revealed 46 nm individual tubulin subunits within protofilaments. STM and related techniques (atomic force microscopy, scanning near field optical microscopy, scanning ion microscopy) offer unique opportunities for the study of neuro­ molecular structures.

472.14


In the present study, we attempted to clarify whether cholinergic receptor density is altered in normal aging and whether this is reflective of the resolution needed to detect changes in the kinetics of ACh release during behavior. WGA-HRP was administered to rats and the microdialysis probe that had been previously used for collection, allowing the anatomical localization of neurons of origin. Directly isolated periventricular onset neurons were localized in various areas of the basal forebrain known to contain ACh cells. Subsequent to dialysis, the probe was raised and maintained with a 28 M NaOH buffer (pH 8.0 Tris buffer). The enzyme was then injected with a CMA/100 microinjection pump (50-100 nl at a rate of 1-2 nl/min). Brains were dissected and sectioned for HRP reactivity (TMB histochemistry). HRP filled neurons were localized in various areas of the basal forebrain known to contain ACh cells. In the present study, we examined the [3H]HCh-3 binding sites that were specific for [3H]HCh-3 in bovine membranes exhibited sodium-dependency and was inhibited by HCh-3 and choline with an IC50 of 5.9 nM and 69 µM, respectively. The [3H]HCh-3 binding site was solubilized by 0.2% deoxycholate and 0.025% NaCl. The addition of 5% percent glycerol improved stability, and the binding activity remained constant up to one month when kept at 40°C. The solubilized carrier was applied to a DEAE-Sepharose column and the detergent was exchanged to 0.01% Tween 80. Protein was eluted by a linear gradient of NaCl, and the peak of binding activity was found in the fractions containing approximately 400 mM NaCl. The resin was then washed extensively and binding activity was eluted with a wash solution containing buffer. The basis of the amount of protein loaded on the affinity column and the amount recovered in the eluant was associated with [3H]HCh-3 binding; this step achieved a several thousand-fold purification.

472.10

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472.15 MODULATION OF CORTICAL ACETYLCHOLINE RELEASE BY CHOLINEERGIC AGENTS: AN IN VIVO DIALYSIS STUDY. J. Richard, D.M. Arango and R. Quirion. Douglas Hospital Research Centre 6675 Latrobe Blvd Verdun, Quebec, Canada H4H 1R3.

Recent evidence has suggested the presence of positive (nicotinic) and negative (muscarinic M₂) autoreceptors controlling the synthesis and release of acetylcholine (ACh) on cholinergic neurons in cortex and hippocampus. However, most of these experiments have been performed using in vitro preparations. The aim of the present study was thus to investigate if similar regulatory mechanisms can be demonstrated in vivo. Anesthetized male Sprague-Dawley rats (230-250g) were stereotaxically implanted with a transcardial dialysis probe (cut off 10,000; one to two days later, the end of generation ACh (in presence of 75µM physostigmine) was evaluated in non-moving animals. ACh levels were determined using a gas chromatography/mass spectrometric assay (PCI). Nicotine (1-3ng/kg) stimulated ACh release although not as potently as high K⁺ (100mM) (2-3 fold over baseline). More interestingly, the combination of nicotine (to stimulate the positive autoreceptor) and atropine (to block the negative autoreceptor) was extremely potent in inducing the release of ACh (up to 10 times over baseline). This clearly shows that autoreceptors are present and active in vivo on cortical cholinergic neurons. It also demonstrates the tremendous capacity of these neurons to release ACh following appropriate stimulations (MRC, Canada).

472.16 ACETYLCHOLINE CONTENT AND COMPARTMENTATION IN RAT CORTICAL SYNAPTOSOMES AFTER IN VIVO EXPOSURE OF BASAL FOREBRAIN CHOLINEERGIC NEURONS TO QUINOLINIC ACID. R.H. Metcalf, D.L. Ridell* and R.J. Boeger. Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

In this study, alterations in the concentration and subcellular distribution of cortical synaptosomal acetylcholine (ACh) after QUIN injection into the nucleus basalis magnocellularis (NBM) was investigated. Rats injected with either 600 or 1000 moles of QUIN were assayed for their synaptosomal ACh content at 0.5 hr or 3 hr post-injection. ACh concentrations in the S₂ fractions determined by gas chromatography-mass spectrometry. ACh concentration in the P₃ fraction of normal animals was 25±8 pmol/g protein. ACh in the S₂ fraction ± 10% was recovered in the P₃ fraction and 24±1% was recovered in the S₁ fraction. In comparison to naive rats, cortical synaptosomal ACh was decreased sharply in rats treated with 1000 moles QUIN. As well, a shift in the subcellular distribution of ACh to the cytoplasmic fraction was seen with a 1000 nmole dose of QUIN. These results show that QUIN stimulation of NBM cortical projection neurons, produce both dose-dependent and time-dependent changes in synaptosomal ACh concentrations and subcellular distribution. Conclusions: The NBM serves as an important source of cortical ACh; QUIN induces a shift in the subcellular distribution of ACh; and QUIN-induced alterations in cortical ACh concentrations may be important for the treatment of cognitive deficits.

472.17 CHARACTERIZATION OF N-METHYL-D-ASPARTATE-MEDIATED ACETYLCHOLINE RELEASE IN RAT MEDIAL SEPTAL AREA. L.M. Nishimura* and R.J. Boeger. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Release of acetylcholine (ACh) in the medial septal area (MSA) may originate from collaterals of cholinergic septohippocampal neurons. Receptors for N-methyl-D-aspartate (NMDA) have been located on these septohippocampal neurons and NMDA has been shown to stimulate the release of ACh in several areas of the central nervous system, thus NMDA may have an effect on ACh release in the MSA. Because of the presence of the pentamine toxin in this area suggests an additional role for galanin in modulating septohippocampal activity.

The objectives of this study was to characterize the NMDA-mediated release of ACh from slices of rat MSA. NMDA evoked the release of H₂ACh from superfused slices of the MSA in a dose dependent manner, with an apparent ECSO value of about 100 µM. This NMDA-induced release was significantly reduced by both NMDA-1 (100 nM) and galanin (500 nM). Tetrodotoxin (500 nM) was demonstrated to have an inhibitory effect on the NMDA-induced H₂ACh release from septal slices as well. These findings indicate that the ACh release in rat MSA is mediated through NMDA receptor stimulation and is modulated by galanin. In addition, ACh release in rat MSA appears to originate from axon collaterals of the septohippocampal pathway. This work was supported by Medical Research of Canada.


Because of the limit of sensitivity conventional methods require the use of physostigmine (PHY) for determination of acetylcholine (ACh) release by microdialysis in the brain. Using a sensitive and specific radioimmunoassay, control mechanism of ACh release was studied in male Wistar rats instrumented with microdialysis probe in the striatum under anesthesia with ether and α-chloralose. Drugs were dissolved in artificial cerebrospinal fluid and perfused at a rate of 2 ul/min. Basal ACh release in the absence and presence of PHY (10 µM) was 5.6 ± 0.6 and 49.3 ± 5.7 fmol/min (n = 10), respectively. ACh release was not affected by the addition of atropine (0.1-10 µM) in the absence of PHY while it was increased in the presence of PHY. Pirenzepine (0.01-1 µM) increased ACh release while AF-DX 116 (0.01-1 µM) had no effect on ACh release. These results indicate that auto-inhibition of ACh release operates only under specific conditions where local ACh concentration is elevated extremely, and that presynaptic M₄ receptor is involved in the control of ACh release.
CONTROL OF POSTURE AND MOVEMENT VIII

473.1  DIFFERENCES BETWEEN CEREBRAL PALSY AND CONTROL CHILDREN ON KINEAMIC VARIABLES IN A DRAWING TASK. Hanneke van Mier, Wouter Hulshof & Paul Weerding (SPON: M. Clare). Nijmegen Institute for Cognition Research and Information Technology (NIC), University of Nijmegen, Nijmegen, The Netherlands.

Many tests for the assessment of movement disorders contain a few writing or drawing tasks. Usually measuring the performance on these tasks can only be done by judging the quality of the lines drawn on paper. Recording of the pen movements by means of an XY-tablet (digitizer), provides more objective measurements of drawing quality -errors, curvature of the lines, etc.- and adds many kinematic movement parameters, like velocity, pen pressure, pauses, etc.

In order to assess the possible contribution of these kinematic variables a group of 15 children with cerebral palsy (CP) and a matched control group of 12 normal children were given a number of drawing tasks. These tasks consisted of drawing between two straight or curved parallel lines, making zigzag horizontal and vertical movements connecting small squares, and a Fitts' type of task in which small circles had to be connected to a target, differing in size, distance and direction. In all tasks speed of drawing was stressed.

Large differences between the two groups were found in nearly all kinematic variables. Particularly striking were the results on a "fluency"-measure, i.e. the number of changes (maxima) in velocity, in the duration of the pauses between the drawing of successive individual lines, and in the number of stops. Kinematic variables helped to differentiate between CP-children whose drawing results on paper looked very similar.


We have tested the hypothesis that the desired movement acceleration and deceleration characteristics determine the properties of the phasic muscle activation driving movement.

Normal human subjects performed the following movements about the elbow: 1) step tracking movements of different amplitudes and durations, 2) cyclic movements of different amplitudes and frequencies, 3) phase plane tracking movements of different acceleration durations and 4) cyclic isometric movements of different frequencies and peak forces.

EMG burst durations and acceleration durations ranged from 100 to 500 ms. For each movement type the duration of the phasic EMG activity initiating movement varied linearly with acceleration duration. Across all movements/sbects the slope of the regression line was approximately 1.

The data show that relations exist between muscle activation and movement properties which are independent of movement type. Thus, the CNS can utilize knowledge of the desired acceleration duration in determining appropriate duration of phasic muscle activation to initiate movement.
473.5


To determine whether community-dwelling elderly fallers (N = 15; mean age = 75.0 ± 4.3 y) differ from elderly nonfallers (N = 12; mean age = 75.0 ± 4.9 y) in speeded cognitive processes underlying preparation and anticipation of reaching movements, both subject groups were assessed with respect to upper extremity movement for Reaction Time (RT) and Movement Time (MT). A paradigm was used in which 75% of the trials had precues that permitted anticipatory planning of the remaining 25% that had invalid precues (i.e., differed from target stimulus), thus permitting examination of the pre-cue effects to the performed response. Trials consisted of one of four precue stimuli presented on a computer screen for 200 msec, followed by a performance interval of 875-1000 msec, and then a target stimulus for which subjects responded by manually pressing a corresponding button. Fallers had slower mean total (RT + MT) performance times for valid precues compared with nonfallers (1041 msec vs. 1016 msec, respectively), suggesting a lack of benefit for motor planning from the precue in fallers, but demonstrated faster mean total times for invalid precues (1183 msec for nonfallers), implying that the motor plans of fallers required less alteration, presumably because they were less well established.

The interaction between subject group and precue stimulus was significant (p < .05). We interpret these results to indicate that fallers have reduced cognitive ability to use relevant contextual information in the planning of simple movement tasks.

Supported by NIH Grant AG08615

473.7

AN INVESTIGATION OF POSTURAL CONTROL STRATEGIES: THE INFLUENCE OF MATURATION. E.M. Earl* and J.S. Frank. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Research into the control of human posture has shown that consistencies are present in the responses and adaptations to compensations for internal and external perturbations (Cordo and Nashner, 1982). The purpose of this study was to investigate the influence of maturation on the reactive and anticipatory control of posture. Children, 4-8 years of age (n=17), were asked to perform voluntary plantar flexion (i.e., heel to floor) to react externally generated handle translations. EMG and kinetic data were collected to document the compensatory adjustments made to cope with these disturbances. Maturation was found to enhance postural control.

The frequency of anticipatory muscle activation increased as development progressed. Maturation was also associated with shorter latencies, and improved temporal coupling of the postural muscles during compensation for both types of perturbations. Although the children used a variety of muscle synergies to cope with both types of disturbances, the horizontal ground reaction forces were found to be consistent for both the internal and external perturbations. (Supported by NSERC)

473.8


It is well-documented that older adults are slower than younger adults, particularly in performing tasks that require the selection of a given movement over another, that is, Choice Reaction Time (CRT) tasks. According to Welford (1973), this response slowing occurs under conditions of uncertainty, and is due to the requirement to select a particular movement over another, and not simply due to slower movement execution.

The present study was designed to investigate this problem by using a bimanual "push or pull" task. Eight older subjects (70-80 years) and 8 young subjects (20-24 years), all healthy and free of neurological disease, participated in the study. Subjects were required to react to a visual stimulus as fast as possible. Measures of response latency as well as EMG onset of relevant muscles were taken.

Results obtained showed that the older group selectively activated the tibialis anterior or gastrocnemius muscles (primary ankle stabilizers for push and pull movements, respectively) with the same latency as the younger subjects. Despite the similar muscle activation times, older subjects showed considerably longer movement latencies with increased response uncertainty. These results suggest that the slower response times observed in the uncertainty conditions were not due to processing deficiencies in determining the movement, but rather to deficiencies in the planning and/or implementation of the motor action. Additionally, the older group showed a differential pattern of activation of the musculature associated with anticipatory postural adjustments. Such differences may also be a detrimental effect on the implementation of voluntary movement in aging populations.

474.1


We investigated the influence of cognitive set on long-loop responses in normals subjected to rotational perturbations of various amplitudes. EMG recordings were obtained from 3 muscles in the left leg in 10 normal adults (age range = 25-41, mean 34.3; 5 females, 5 males). Stimulation consisted of three sets of 20 trials of sudden toe-up ramp movements of pre-selected amplitudes (pure 4°, pure 10°, and mixed 4°/10°) at a constant velocity of 35 degrees/sec. For the pure 10° displacement, long-latency responses showed no significant differences. Mean normalized long-latency tibialis anterior (TA) amplitudes of both the pure and mixed 10° displacements were increased in comparison to the pure 4° displacements. Randig &4° TA mean amplitudes were similar to random and pure 10° responses. We concluded that scaling of the TA long-latency response, and not changes in response latencies, represents the major postural adjustment mechanism in this population, and favors a large response in situations where perturbation size cannot be reliably predicted in advance.

474.2


The activation of motor neurones in a muscle pool is said to proceed as an ordered array in response to both increasing dorsal root stimulation and voluntary activation, with small motoneurones being recruited before larger ones. We have examined 19 voluntarily recruited soleus motor units in 5 human subjects and found that in 18 cases the lowest threshold motor unit recruited by voluntary activity is different from the lowest threshold unit recruited by electrical stimulation of the tibial nerve (Hoffman reflex). These low level voluntary units are influenced by the stimulated fibres as, during tonic activation, their firing interval remains far smaller than that required for recruitment at rest. These findings suggest that the pool of soleus motoneurones responding to the voluntary command “plantar flex your ankle” differs, in order of activation, from the pool responding to stimulation of the largest diameter fibres in the tibial nerve. This could be explained by partitioning of excitatory input to the soleus motoneurone pool: either of cortical input or of IA afferents.
474.3


During ballistic movements there is too little time for conscious modification of motor programs in response to proprioceptive input. We examined rapid flexion movements of the elbow for evidence of reflex activation in response to unexpected extensor movement moment during the movement.

Trained, rapid (peak velocity 467 m/s deg/sec Mean±SD), accurate, flexion movements were interrupted by applying opposing 6 Nm torque in 12 subjects who were unaware of such a possibility. During training, subjects developed an EMG signature for each movement. The earliest change in this signature after a torque pulse occurred at a mean of 248 ms which is similar to voluntary reaction time. Some subjects reproduced their entire movement signature, including inappropriate triceps activation, despite movement reversal by the torque. No evidence of a short latency compensatory response was seen. These findings suggest that in naive subjects there is no short-latency mechanism for modification of inappropriate ballistic movements once they are initiated.

474.5

EFFECTS OF LOAD-CARRYING ON MUSCLE ACTIVATIONS AND MOVEMENTS OF THE LOWER EXTREMIT Y DURING normal locomotion. E. A. Keshner and B. W. Peterson. Neurobiology Lab. and Dep't, of Physiotherapy, Fac. of Med., Laval University, Quebec, Canada.

We studied the effects of load-carrying (with a weighted jacket) on muscle activations and movements of the right leg during stair ascent. Six normal subjects, aged 25 to 47 years, performed 3 tests consecutively: 1) unloaded, 2) with a 16 Kg load, 3) with a 22 Kg load. During tests, sagittal movements (TRAX electromyometer) and EMG activity (surface electrodes) from 5 muscles (vastus medialis; rectal hamstrings; medial gastrocnemius; tibial anterior) were recorded. Temporal parameters of the stair ascent cycle (cycle duration, % stance and swing) were

474.4

474.6


The task of maintaining equilibrium in a bipedal stance becomes more difficult as a function of the size of mass displacement relative to the width of the base of foot support. In this study, we examined the effects of stance width and perturbation on kinematics and EMG patterns in response to support surface displacements in the lateral direction. As stance width decreased or displacement size increased, muscles engaged in a more rapid trunk lateral flexion with hip abduction/adduction to correct posture. These kinematic changes from an erect trunk to a flexed trunk were associated with an increased magnitude of EMG bursts but no changes in EMG temporal patterns or onsets. Reciprocally activated proximal trunk and hip muscles were always activated prior to cocontraction of distal ankle muscles. The magnitude of initial EMG bursts in both proximal and distal muscles were scaled to the size of surface displacements although bursts were initiated prior to the time information on displacement size was available to the CNS. These results indicate that as the postural task becomes more difficult, trunk lateral flexion increases due to increased muscle burst magnitudes based on current stance width and on prior experience with displacement size, without a change in the EMG pattern. (Supported by CIDA 881094, 801 AG6462, and MHC of Canada.)

474.8

INHIBITION OF AN HOMONYMOUS MONOSYNAPTIC, BUT NOT AN HETERONYMOUS OLigosynaptic SHORT REFLEX IN THE HUMAN LEG DURING WALKING. D. F. Collins*, J. D. Brooke, and E. R. McIlroy. School of Human Biology and Biophysics Group, University of Guelph, Ontario, N1G 2W1, Canada.

Short latency reflexes are modulated by the level of ongoing contraction in the target muscle. At similar levels of contraction, Soleus (Sol) H reflexes are depressed during walking, compared to standing. Currently, we compared the movement of a non-nonsynaptic monosynaptic reflex to that of a previously identified heteronymous oligosynaptic reflex which involves two joints of the limb. H-reflexes were evoked in the Sol muscle during walking and standing, at equal stimulation intensities (as % Mmax of Sol) and over a range of contraction levels. The H Reflex magnitude was significantly depressed in the walking condition, compared to standing, at similar levels of contraction. The H reflex excitation was evoked in the vastus medialis (VM) muscle by stimulation of the common peroneal nerve at caput fibula. No significant difference was identified between the VM reflex magnitudes during walking and standing at equal stimulation intensities (as % Mmax of Sol) and over a range of contraction levels. This selective inhibition of the monosynaptic reflex exposes differences in the central control of these two segmental pathways during such movements. Supported by grant from NSERC Canada #A9005.
474.9 THE CONDITIONING EFFECTS OF CUTANEOUS STIMULATION ON THE HUMERAL H-REFLEX DURING STANDING AND WALKING. L Furutani and M. Bach, School of Physical & Occupational Therapy, McGill University, Montreal, Quebec H3G 1Y5.

The modulation of the soleus H-reflex by a conditioning cutaneous stimulation was investigated in normal subjects during standing (n=9) and treadmill walking (n=2). The test H-reflex was obtained by stimulating the tibial nerve in the popliteal fossa using a single 1 ms pulse. The conditioning stimulus, corresponding to a maximum and stable H-reflex was first determined in standing. During walking, the effective stimulus strength was controlled by varying the intensity to obtain a stable M response similar to that observed in standing. Conditioning stimulation corresponded to an 11 ms train of three 1 ms pulses at 200 Hz, delivered to the sole of the foot. The intensity was varied from sensory threshold (IT) to maximal (max T), with the flexion reflex in the tibialis anterior muscle monitored.

During standing, conditioning-test delays between 15 to 100 ms were given randomly. A conditioning cutaneous stimulation of max T resulted in a marked inhibition of the H-reflex amplitude (0% - 20% control) at 45 ms delay, and a late facilitation (200% - 300% control) at 100 ms delay. The H-reflex was not modulated with a conditioning cutaneous stimulation of IT.

During walking, when tibial nerve stimulation was given alone, the H-reflex was modulated throughout the gait cycle, such that the amplitude was increased from midstance to push off, and inhibited in swing phase. When a conditioning cutaneous stimulation of max T resulted in a marked inhibition of the H-reflex amplitude (45 ms delay), and a late facilitation (100% - 300% control) at 100 ms delay. The H-reflex in the swing phase remained completely inhibited for all intensities.

These results confirm previous observations on the task-dependent modulation of the H-reflex, and ininsic to the stance phase of gait can be progressively reversed by a conditioning cutaneous stimulation of increasing intensity. (Supported by the MRC)

474.11 A KINETIC ANALYSIS OF PERTURBED LANDINGS. H. Sveis'trup, A.T. Hoshizaki, (SPDN) B. McFadden, Biomechimics Lab., MCGILL University, Montreal, Quebec, Canada, H3G 1Y5.

Two women were unexpectedly dropped from a height of 18 cm and exposed to an 8 cm backward perturbation of the support platform at different delays following landing. The perturbation conditions were none, short delay (0.1 s), medium delay (0.2 to 0.3 s), and long delay (0.4 to 0.5 s). Cine data were collected (100 Hz) and lower limb joint angular kinematics evaluated. Pearson product-moment correlation coefficients calculated between trials with conditions were used to determine consistency in the shapes of the joint angular response curves. The range of the correlation coefficients collapsed across conditions for each subject was 0.80 to 0.99 for the ankle, 0.84 to 0.99 for the knee and 0.00 to 0.99 for the hip joint. The range of correlation coefficients for subject 1 was 0.60 to 0.98 for the ankle, 0.40 to 0.98 for the knee and 0.13 to 0.98 for the hip joint. For both subjects, the ankle and knee joints displayed consistent kinematic patterns while the hip joint was more variable indicating that the kinematic patterns remained fairly stable even though different neuromuscular strategies may have been used in response to the different task demands. H. Sveis'trup is partially funded by FGAR, Quebec.


The effects of a static contraction on torques generated at an adjacent joint was investigated in normals. A dynamometer was used to measure isometric torques exerted simultaneously in flexion/extension, abduction/adduction and internal/external rotation at the hip and flexion/extension at the knee. This dynamometer was interfaced with a desktop computer which was used to display the generated forces and torques of a specific joint.

Subjects (n=4) were required to successively exert flexion and extension of the hip or the knee at 30% of the maximal voluntary contraction (MVC). Subjects were instructed to perform flexion and extension of the hip without feedback regarding torques in abduction/adduction and internal/external rotation of the hip and torques generated at the knee. Similarly, flexion/extension of the knee was controlled while no feedback was available on torques exerted at the hip.

Results show that subjects perform extension of the hip at 30% of MVC with an associated contraction of the knee probably reflecting the use of the hamstrings muscles. In contrast, either flexor or extensor torque at the knee are observed with hip flexion. A flexor torque exerted at the knee results in a flexor, abductor and internal rotator torques at the hip. Extension of the knee causes a substantial extensor torque at the hip among all subjects evaluated.

It appears that associated torques are present in normals and that these torques are greater during the production of a distal torque at a uniaxial joint than during exertion of a multiaxial torque. Our study is ongoing to quantify and compare these associated torques in both normal and hemiparetic subjects. (Supported by Health and Welfare Canada HRC and FR$Q)

The transition from stationary standing to walking is initiated via a deactivation of the ankle extensor muscles followed by ankle flexors, which presumably elicits and reinforces a forward acceleration of the body center of mass prior to the removal of the stepping leg from the ground.

The ankle muscle timing pattern was examined prior to heel-off (HO) of the initial step with respect to possible differences for control strategies for self-paced (SP) vs. externally triggered (ET) movements, and in interlimb responses related to upcoming swing and stance functions. Subjects (n=5) stood on a force platform and executed a series of steps under ET (light-flash) and SP conditions. Surface EMG was recorded from soleus (SOL) and tibialis anterior (TA) muscles bilaterally, while a foot switch detected the initial HO. The three principal findings emerged: (1) an offset of tonic SOL activity always (pc.05) preceded TA activation; (2) generally enhanced rectified (SOL) or offset (TA) for SP vs. ET movements at comparable stepping speeds was observed, while intersubject variability was considerably greater for the former; (3) The intramuscular timing interval (SOL-TA) was longer (pc.05) for the upcoming stance vs. swing leg, and may reflect the need to unload the latter while continuing the forward acceleration of the body.

OCULOMOTOR SYSTEM IV


The superior temporal polysensory area (STP) is a prefrontal cortex in the upper bank and fundus of the rostral superior temporal sulcus, which has extensive cortical connections and single unit response properties, we suggested they might play a role in visual orientation and eye movements (Bruce et al., 1982).

The purpose of this study was to determine the role of STP in oculomotor control by removing this area in monkeys and examining the influence of small, medium and large saccadic eye movements to visual targets that appeared 8, 15, and 25 degrees away from the fixation point, above the fixation level and 8 degrees above and below the fixation point. Smooth pursuit and 20 degrees per second, three of four pairs of targets, in an increase in saccadic latency of 30 msec to the most peripheral contralateral target and smaller saccadic latency increases to less peripheral targets. The unimpaired animal had the smallest lesion. Recovery latency increased to prepulse intervals of 3 to 5 weeks after surgery. Saccadic accuracy was unimpaired by any STP lesion.

In contrast with the saccadic latency increase, saccadic lesions did not produce any smooth pursuit deficit. In addition, the saccadic latency deficit does not reflect a general oculomotor or motivational deficit, but rather an impairment in visual orientation.

CHANGES IN JOINT POSTURE AND CENTER OF MASS LOCATION DURING ABRUPT PULLS OF DIFFERENT FORCES MADE BY STANDING HUMANS. W.A. Lea, C.F. Michaud* and T.C. Pat. Physical Therapy, Northwestern University, Chicago IL 60611.

Bernstein (1967) hypothesized that the control of complex actions is simplified by the adoption of invariant movement synergies that are scaled to the speed or force of the action. Alternatively, different movement patterns might be used for actions with different dynamics. The purpose of our study was to determine if a single movement synergy characterizes bilaterally symmetrical, abrupt pulls in the sagittal plane made by well-practiced standing humans.

Hip, knee and ankle joint angles, and the locations and velocities of the center of mass (CM) in the anterior/posterior (AP) and vertical (V) directions were computed from data collected with a WATSMART system from 3 well-practiced subjects who pulled at 2, 10, 20, 40, 60, 80 and 95% of maximal pulling force (%MPF). Overlaid time records, angle-time diagrams and CMAPs vs CM plots were used to determine if movement patterns were invariant. Linear regression assessed the extent to which the peak change in each variable was scaled to %MPF.

The onset, peak, and offset times of changes in each variable were relatively constant. Only the peak change in CMAP, CMAP velocity and ankle angle were significantly correlated with %MPF in all three subjects. Little or no change occurred in any variable at 5 or 10% MPF. All subjects' CMAPs moved backward before pulls 10-25% MPF. CMs decreased during forward pulls >50% MPF. All subjects showed ~5% MPF pulls for Subj. 1. Subj. 1 rotated back, plantarflexing the ankles and flexing the knees and hips before 10-20% MPF; >40% MPF; the combined movement of ankle dorsiflexion, knee and hip flexion followed by ankle plantarflexion (other angles constant), with hip and knee extension ("drop and thrust up"). Subj. 2 plantarflexed only the ankles before 10-20% MPF pulls ("rotate back, body straight"), concurrently plantarflexed the ankles and flexed the hips at 40-80% MPF ("jack-knife back"); and flexed all three joints at 95% MPF. Subj. 3 backward movements occurred almost exclusively at the ankle, but some concurrent hip and knee flexion occurred above 60% MPF.

We conclude that subjects alter their movement patterns as pulling force increases, which argues against the hypothesis that one movement synergy underlies bilaterally symmetrical abrupt pulls. The variations in knee, hip and ankle angle and CM movement patterns show that subjects have some flexibility in organizing the kinematic degrees of freedom of the task.
475.3 OCULOMOTOR DEFECTS ASSOCIATED WITH LESIONS OF THE FRONTAL EYE FIELD AREA IN MACAQUE MONKEYS. M.G. MacAvoy and C.J. Bruce. Dept. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510. We studied horizontal and vertical saccades in 10 patients with unilateral lesions of the FEF and 10 age-matched normal subjects using the double-step paradigm (DSP) and remembered targets (RT). Subjects also followed a target which alternated between two horizontal positions at progressively shorter intervals (2.0 - 0.1 sec; AT). We found that the effective lesions included the restricted area in and near the fundus of the arcuate sulcus where microstimulation elicits smooth, usually ipsilaterally-directed, eye movements (MacAvoy, Soc. Neurosci. Abstr. 1988), in contrast to contralateral saccades readily elicited from sites of FEF. We analyzed this smooth pursuit tracking deficit with sinusoidal, linear, and step ramps. Sinusoidal tracking was very asymmetric; all frequencies tested (0.25-2.5Hz) had reduced smooth pursuit velocity and a preponderance of catch-up saccades for the ipsilateral direction. Similar deficits obtained with linear tracking, with low gains even at low target velocities, even though smooth velocities up to 35°sec were obtained with a 75°sec target. This directional deficit was present in response to step-ramp target motion in both the left and right visual fields. Ipsilateral saccadic eye movement size and latency in conjunction with ramps were also affected. Predictive aspects of oculomotor behavior were drastically affected, especially eye movements in the target motion in the effective lesion. Smooth pursuit tracking precentripetal target motion was almost abolished for ipsilateral directions. Predictive continuation of sinusoidal tracking following target extinction was similarly affected. Predictive saccadic tracking was also reduced. Supported by PHS grants EY04740, NS22807, MH48866.

475.4 SACCADES IN HUMANS WITH LESIONS OF FRONTAL EYE FIELD (FEF) W.A. Fletcher*, R.E. Gellman*. Dept. of Clin. Neurosc. and Med. Phys. Univ. of Calif., San Francisco, CA 94143. We studied horizontal and vertical saccades in 10 patients with unilateral lesions of FEF and 10 age-matched normal subjects using the double-step paradigm (DSP) and remembered targets (RT). Subjects also followed a target which alternated between two horizontal positions at progressively shorter intervals (2.0 - 0.1 sec). Mean saccadic latency for right and left RT and horizontal accuracy and peak velocities for all target conditions were normal. However, saccadic latencies for RT were abnormal in 8 patients. Prolongations of latencies were greatest for contralateral targets and in the direction of the lesion, 1 had bilateral prolongation and 4 had subnormal latencies due to excessive predictive saccades. For RT, the minimum intersaccadic interval preceding contralateral saccades was probably in functional areas 7a/LIP, MST, peripheral V2 and the superior temporal polysensory area. Horizontal saccades to moving targets, and predictive eye movements, both saccadic and smooth, were normal. However, saccadic latencies for RT were abnormal in 8 patients: 3 had prolonged latencies up to 500 ms before motion commenced on the ipsilateral direction. Similar deficits obtained with linear tracking, with low gains even at low target velocities, even though smooth velocities up to 35°sec were obtained with a 75°sec target. This directional deficit was present in response to step-ramp target motion in both the left and right visual fields. Ipsilateral saccadic eye movement size and latency in conjunction with ramps were also affected. Predictive aspects of oculomotor behavior were drastically affected, especially eye movements in the target motion in the effective lesion. Smooth pursuit tracking precentripetal target motion was almost abolished for ipsilateral directions. Predictive continuation of sinusoidal tracking following target extinction was similarly affected. Predictive saccadic tracking was also reduced. Supported by PHS grants EY04740, NS22807, MH48866.

475.5 UNIT ACTIVITY RELATED TO SMOOTH Pursuit EYE MOVEMENTS IN Rhesus Monkey FRONTAL eye fields J.P. Gottlieb*, M.G. MacAvoy and C.J. Bruce. (SPON: D. Burman) Section of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510. Single unit activity was studied within and near the region of the f rontal eye fields (FEF) where smooth eye movements are elicited by microstimulation (MacAvoy et al., Soc. Neurosci. Abstr. 1988). 44 neurons responded to linear (constant velocity) or sinusoidal smooth pursuit tracking tasks, but had no (or minimal) responses during visually-guided saccade tasks. All neurons were tested for pursuit directions and the responses began 90-600 msec after target movement onset. During linear tracking most neurons had tonic responses lasting throughout the track - 2s or longer; others responded for only 3-400 msec. Activity during sinusoidal tracking was extinguished following the period of constant velocity pursuit and eye movements. Most neurons were also tested while the monkeys fixated a stationary light during trials on which the monkey could expect centripetal motion (linear or sinusoidal) in the neuron's ON direction a response still followed, as if anticipating the reversal. 3) Movements. Supported by PHS grants EY04740 and NS22807.

475.6 FEEDBACK FROM NATURAL SACCADES PERTURBS THE DIRECTION AND AMPLITUDE OF SACCEADES ELICITED BY MICROSTIMULATION IN THE MONKEY'S FRONTAL EYE FIELDS. Charles J. Bruce and Gary S. Bizarre, Sect. Neuroanatomy, Yale Univ. Sch. med., New Haven, CT 06510. Saccades are coded topographically, across the primates front al eye fields (FEF), with each location representing a particular direction and amplitude; however, Schlag and Schlag-Rey (1987, 1996) recently reported that the memories of saccades electrically elicited from the FEF can be dramatically modified if the stimulation coincides with a natural saccade. We further examined this phenomenon, testing FEF sites yielding saccades as a low thresholds (< 50 µA). The monkeys began each trial by fixating an eccentrically-located target. After ~1 sec the target jumped to the center of the monitor, and the monkey's ensuing saccade automatically triggered a 20°ms train of electrical stimulation within ~10 msec of the saccade onset. During this stimulation usually elicited another saccade that began after the natural one ended. By varying the location of the eccentric fixation target, we analyzed the effect of the dimensions of the natural saccades upon the dimensions and latencies of the electrically elicited ones. Our results confirm those of Schlag and Schlag-Rey in that the elicited saccades were systematically modified; however, saccade dimensions were not simply translated by the translaminar FEF vectors to craniotopic goals. Instead, we hypothesized that the modified elicited saccades reflect a partial vectorial subtraction of an effector copy of the preceding natural saccade from the characteristic saccadic vector of the stimulation site. In the natural situation this subtraction serves to cancel FEF activity immediately after it is used, thereby preventing multiple saccades. Moreover, it also provides a non-'spatial' explanation for oculomotor behavior in the double-step paradigm of Hall & Lightstone, and thereby alleviates much of the motive for postulating a craniotopic stage of saccadic processing. PHS Grants EY04740 & NS22807.

475.7 TOPOGRAPHICAL PROJECTIONS FROM THE FRONTAL EYE FIELDS TO PARIETAL, TEMPORAL AND OCCIPITAL CORtical AREAS IN THE MACAQUE MONKEY. C. G. B. Swanton, M. E. Goldberg, and C. J. Bruce, Dept. of Anatomy, Howard Univ Coll. of Med., Washington, DC 20059, Lab. Sensorimotor Res., Natl Eye Inst., Bethesda, MD 20025, and Section of Neuroanatomy, Yale School of Med., New Haven, CT 06510. Frontal eye field (FEF) projections to posterior cerebral cortical areas were studied with autoradiography in four macaque monkeys. Injections of tritiated amino acids were placed into large (IEFE) and small (sFEF) saccade sites within the physiologically defined FEF. Labeling was usually densest and most widespread in layer I and moderate to dense in layers V-VI with hot spots of labeling in all layers. Hot spots for IFEF projections were (causal to rostral) the anterior lip of the posterior parietal lobule (PFL), superior bank of the superior temporal sulcus (STS) and posterior cingulate gyrus. Light labeling was seen on the superior lip of the calcarine sulcus. The IFEF labeling was probably near MST, V2, V3a, Vd, V4. V4 were probably labeled from sFEF. These results suggest that the FEF are topologically organized with respect to visual and visuomotor areas of the posterior cortical areas. Supported by NIH grant EY03763.

475.8 INTERACTIONS OF VISUAL AND MOTOR-PLANNING ACTIVITIES IN THE LATERAL INTRA-PARIETAL AREA (LIP), S. Burghard, R.M. Baekgaard, L. Forssvik, and R.A. Andersen, Dept. of Brain and Cognitive Sciences, Rm E25-206, M.I.T., Cambridge, MA 02139. We further investigate the role of macaque cortical area LIP in visual-motor integration. 1. Detailed quantitative analysis of 145 LIP neurons revealed: (1) Activity during delayed saccade trials typically has 3 distinct phases related to target presentation (TP), eye movement (EM) and target completion (TC). (2) LIP neurons are involved in the generation and control of smooth, as well as saccadic, eye movements. Supported by PHS grants EY04740 and MH48866.
MICROSTIMULATION OF A NEURAL NETWORK MODEL THAT COMPUTES COORDINATE TRANSFORMATIONS FOR VISUALLY GUIDED SACCADES. R.A. Andersen and J. Schlag-Rey. Dept of Neurology, Johns Hopkins Hospital, 600 North Wolfe St., Balt. MD, 21205.

When brain areas are microstimulated at different initial eye positions, three types of eye movements and visual responses were evoked. We have found different types of dual pathways in the primate brain, which suggests that the output of the FEF represents a saccade goal in retinal coordinates, rather than a motor command. This goal is later translated into spatial coordinates.


475.11 THALAMIC CONNECTIONS OF MONKEY SUPPLEMENTARY EYE FIELD. B. Shook, M. Schlag-Rey and J. Schlag. Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.


475.15 MICROSTIMULATION OF PRIMATE FRONTAL EYE FIELD SPECIFIES RETINOTOPIC GOAL OF EVOKED SACCADE. P. Dassonville, J. Schlag and M. Schlag-Rey. UCLA, BRI & Dept. of Anatomy, Los Angeles, CA 90024.

The colliding saccade paradigm, i.e., microstimulation during or shortly after an ongoing saccade, can differentiate the roles played by various oculomotor regions in saccade generation. Deep collicular stimulation specifies the saccade vector, whereas superficial collicular stimulation specifies the saccade's retinotopic goal. Thalamic stimulation creates an artificial retinal error signal referred to a previous eye position, with a delay adequate for compensating early visual looming time (Exp. Brain Res., in press). Applied to the frontal eye field (FFF) in 2 monkeys (Macaca nemestrina), the paradigm shows that, as in the thalamus, thalamic stimulation creates a retinal error signal referred to a previous eye position, eliciting a saccade compensatory for both afferent and efferent delays in the oculomotor system. Compensation was seen at 37 stimulation sites regardless of the type of presaccadic unit activity present, i.e., visual, visuomovement, or movement. This suggests that the output of the FEF represents a saccade goal in retinal coordinates, rather than a motor command. This goal is later translated into spatial coordinates. (Supported by USPHS grants EY05879 and EY02305, and NSF grant R01EY-30343.)
475.15

SACCADIC IN PROGRESSIVE SUPRANUCLEAR PALSY (PSP)
W.T. Corbluth*, T.C. Hain, N.R. Miller (SPON W.
Browell). Johns Hopkins University School of Medicine,
Baltimore MD, 21205.

PSP is a progressive disease of humans in which most
damage is found in the globus palli-
dus, subthalamic nucleus, prefrontal and superior
colliculus. In 3 subjects with PSP we measured
saccadic latencies, saccadic amplitudes, and
physiological and ophthalmological parameters.

Horizontal and vertical saccadic latencies were
242 ± 18 ms (range 181-283) in 20 patients
and 204 ± 21 ms (181-231) in 20 patients.

Horizontal saccades velocities declined from 95%
of normal at 1 deg to 20% of normal at 10 deg.
Vertical saccade velocities declined from 53% of
normal at 1 deg to 2% of normal at 4 deg.

The difference in latencies implies separate
initiating mechanisms for horizontal and vertical
saccades. The inability to make large saccades
and reduction in the velocity of saccades is
consistent with the pathologic findings.

475.17

EXCESSIVE ANTICIPATORY SACCADE EYE MOVEMENT IN SCHIZO-
PHRENIA. D. U. Honner and A. D. Redant*. GREEC, VA
Medical Center, Seattle, WA 98108.

We examined smooth pursuit as well as pure saccadic
movements in neuroleptic-treated schizophrenics (n=22),
patients with mixed drug and alcohol abuse (n=20) and
controls (n=27). Significant differences were found in
patients with mixed drug and alcohol abuse (n=20) and
controls (n=27).

In patients with mixed drug and alcohol abuse (n=20),
saccadic latencies were significantly lower than those in
controls (n=27). This suggests that the smooth pursuit
system is impaired in patients with mixed drug and alcohol
abuse.

475.18

POST-SACCADIC DRIFT IN STRABISMICS FOLLOWING BOTULINUM IN-
CHRU, 35000, Nantes, France.

We examined post-saccadic drift in four adult patients
with esotropia (10-40 dioptries) before and after injection
of botulinum in the medial rectus muscle of the.
Saccades were recorded binocularly with the EOG; patients viewed
binocularly horizontal LED targets in all sessions. After
injection, the altered eye was patched. The toxin created
changes in latency and peaked, position-dependent, post-
saccadic drift in that eye within one day. We concentrated on
abducting saccades in the range of action of the non-
altered muscle. The peak latency change of the drifted eye was 25, 7, 305 of the antecedent saccade for the
patients examined 1 day later, and 83% for one patient exam-
ined 4 days later. After 4 hrs of binocular viewing, drift in
the altered eye decreased by 2-10%; however, this in-
duced drift in the non-altered eye by a similar amount.

475.19

PREDICTIVE AND VISUALLY-DRIVEN COMPONENTS OF SINUSIODAL
SMOOTH PURSUIT EYE MOVEMENTS. E. J. Morris* and S. G.
Tubergen* (SPON E. Mayer). Dept. of Psychol., Neurosci. Gradate
Program, Univ. of Calif, San Francisco, CA 94143.

The aim of our study was to determine whether monkeys utilize a
nonvisual predictive signal as a command for eye acceleration during
smooth pursuit of sinusoidally-moving targets. In a previous study (SOC.
Neurosci. Abstr., 11.79) we used cross-correlation analysis to obtain
target functions relating eye acceleration to visual error signals. A
computer model based on these transfer functions accurately simulated
pursuit of targets whose velocity varied sinusoidally and unpredictably.
We have now found that a model based on visual error signals alone
fails to simulate accurately this phenomenon.

In the present study monkeys pursued targets moving sinusoidally over
a range of frequencies and amplitudes. Cross-correlation analysis of eye
acceleration and pursuit velocity revealed a new predictive component of eye
acceleration with a gain of approximately 0.7 in phase with target
acceleration. Components related to visual errors were also obtained having approximately the same gains as those for pursuit of unpredicted target motion; gains of these visual error components were
independent of target phase. Total eye acceleration behavior of the
linear summation of these two components. The model predicts the
predictive component in our computer model greatly improved the
accuracy of its simulations. We conclude that the regularity of sinusoidal
target motion is dependent on the predictive component of eye
acceleration as a feedforward command to improve pursuit performance.
(Supported by NIH Grant EYO3878)

475.20

PRECISION SMOOTH PURSUIT EYE MOVEMENTS REQUIRE
MOTOR LEARNING. S.J. Heintet* and E.L. Keller, Smith Kettlewell
Eye Research Institute, 2332 Webster Street, San Francisco, CA
94115 and Dept. of Elect. Eng., Univ. of Calif, Berkeley, CA 94720.

It has been suggested that the primate's repertoire of eye move-
ments is large and variable, and is not based on motor learning.

In the present study monkeys pursued targets moving sinusoidally over
a range of frequencies and amplitudes. Cross-correlation analysis of eye
acceleration and pursuit velocity revealed a new predictive component of eye
acceleration with a gain of approximately 0.7 in phase with target
acceleration. Components related to visual errors were also obtained having approximately the same gains as those for pursuit of unpredicted target motion; gains of these visual error components were
independent of target phase. Total eye acceleration behavior was obtained having approximately the same gains as those for pursuit of unpredicted target motion; gains of these visual error components were
independent of target phase. Total eye acceleration behavior of the
linear summation of these two components. The model predicts the
predictive component in our computer model greatly improved the
accuracy of its simulations. We conclude that the regularity of sinusoidal
target motion is dependent on the predictive component of eye
acceleration as a feedforward command to improve pursuit performance.
(Supported by a Rachael Atkinson Fellowship and EYO6860)
PARALLEL COMPUTER MODEL OF THE LIMULUS LATERAL EYE. Ramkrishna Prakash*, Eduardo Solesio* and Robert B. Barlow. Institute for Sensory Research, Syracuse University, Syracuse, NY.

The retina of the Limulus eye is the largest neural network for which a quantitative model exists. In a pioneering study, Hartline, Ratliff, and their colleagues formulated a neural network model that describes the steady-state responses of single optic nerve fibers in terms of the properties of single neurons and the interactions among them. Subsequent studies extended the model to include essential nonlinearities and temporal properties of excitation and inhibition. Parallel computers are ideal for modeling neural networks such as the Limulus retina. We have developed a time-dependent model of the retina on the Connection Machine (CM2, 32,500 processor). The model uses 8,192 processors to represent a matrix of 64x128 retinal receptors. We simulate light transduction and adaptation of receptors with the Hodgkin-Huxley model and the interactions among receptors with digital filters.

Connection Machine computations match to first approximation of the patterns of neural activity recorded in the laboratory in response to moving patterns of illumination. We will report how the model responds to changes in the spatio-temporal properties of stimulus patterns as well as to changes in the spatio-temporal parameters of the model itself. The latter are important because we now know that the properties of the retina are modulated from day to night by a circadian clock. Computations with "daytime" and "nighttime" network models will hopefully provide a better understanding of the neural basis of the animal's visual behavior. Supported by NSF 8709059 and NIH grant EY-00667.

PARSIMONY OF NEURAL CONNECTIONS: COMBINATORIAL ADVANTAGE OF GLOBALLY SIZE SCALING INPUT PATTERNS. B. B. Glasmann.

A great theoretical puzzle is how astronomical numbers of neural connections in the brain categorize still larger numbers of input pattern variations. One way may involve size scale restrictions. Consider a hypothetical sheet of N cells. Another neural subsystem, restricted to receiving input from N of these units at a time, must be prepared to read any of C(N,M) input patterns. If the receiving subsystem is further restricted to scaled input patterns whose activated elements are multiples of \( \mathbb{N} \) \( \mathbb{Z} \) elements apart, it must read \( \mathbb{Z}^2/(\mathbb{N}^2/(\mathbb{N}^2)) \) patterns, which is usually a smaller number. This will be illustrated in a family of curves. (The leading multiplier (N) ensures that each of the C(N,M) patterns is read at every possible position. For simplicity, the element sheet is treated here as if it had no edge, or as if N were very large relative to M.) The ratio \( [C(N,M)](1/C(N,M)) \) is the "combinatorial advantage" of size scaling. When both N and M are very large relative to M, the formula \( v^N \) gives the approximate combinatorial advantage.

One use of this line of analysis might be in considering size scaling and connectivity of the multiple cortical mappings of the retina.

476.4 LEARNING RECEPTIVE FIELDS THAT ARE MATCHED TO AN IRREGULAR PHOTORECEPTOR LATTICE. L. T. Maloney* (SPON: R. M. Shapley).

Department of Psychology and Center for Neural Science, New York University, New York, New York 10003.

A method is described that reorganizes receptive fields of a model visual system so as to compensate for irregularities in the photoreceptor lattice. It makes use of visual information in patterned visual scenes, can correct for optical distortions, and could supplement other chemical and electrical cues active in visual neural development.

The method requires that the system is able to compute transformations \( i \) that would compensate for eye movements if the receptive fields in the visual system were matched to the photoreceptor lattice. If the receptive fields and lattice are not matched, the method uses the transformations to generate an error signal by comparing visual input across eye movements. The error signal guides reorganization. The method requires no knowledge of the contents of a particular visual scene, no feedback, other than the error signal itself computes, and could be implemented by many adaptive algorithms. Simulations indicate that the method is little affected by small errors in eye position information.
476.7 SPATIAL DISTRIBUTION OF CHOLINE ACETYL-TRANSFERSASE (CHAT) LABELED CELLS IN THE MACAQUE RETINA, R. W. Rodieck and D. W. Marshak, Department of Ophthalmology, University of Washington, Seattle WA 98195, Department of Neurobiology and Anatomy, University of Texas Medical School, Houston TX 77025.

Whole-mounted macaque retinas were used to determine the spatial density of CHAT immunoreactive cells. Previous work has shown that these cells correspond to the starburst amacrine cells, and that, in primates, almost all their soma lie in the ganglion cell layer. The density profile has the overall shape of a steep-sided volcano, centered on the fovea. The density was about 1200/mm², rises to about 1600/mm² at 1 mm eccentricity, drops rapidly to about 400/mm² at 4 mm, and then gradually decreases to about 100/mm² near the outer limit of the fovea. A log-log plot of the density profile versus eccentricity was fitted by a second order polynomial. The results are consistent with the findings of other laboratories, and suggest that starburst amacrine cell density does not necessarily decline to a very low level near the edge of the fovea.

476.8 DOG RETINAL GANGLION CELLS: MORPHOLOGICAL TYPES AND BREED DIFFERENCES IN TOPOGRAPHY. L. Reichlin, Max-Plank-Institut f. Hirnforschung, D-3550 Marburg, F.R.G.

The morphological types of ganglion cells in the dog retina and their topographical distribution were studied with intraretinal injection of a retrograde tracer with red fluorescent and silver and Nissl staining. Ganglion cells with large somata had large alpha-type dendritic trees, most cells with medium-sized somata had small dendritic trees, and cells with small somata had a variety of dendritic branching patterns. All resembled the types found in cat retinas. Alpha and beta cells divide into inner and outer branching subtypes, presumably representing OS and OFF channel.
746.13
CELLS OF DIGEHL AND GANGLION CELL DISPLACEMENT DURING OPTIC NERVE ISCHEMIZATION, G. L. Simon* and E. S. Bailis. Department of Anatomy and Cell Biology, SUNY-Health Science Center at Brooklyn, Brooklyn, NY 11203.

Cells of Doplil (DC) are retinal ganglion cells (RGC) whose perikarya are normally located among the amacrine cell bodies in the inner nuclear layer (INL) of many species. Their dendritic trees are inserted in the sense of extending vitread into the inner plexiform layer (IPL). We have examined cases of retinal ischemia in rabbits (Baltzer et al., 1981) that some of these cells project to the base optic nucleus in the frog. In a previous study (Sicall et al., 1985), using a flat-mounting technique, we observed massive RGC death in R. pipiens after occlusion of the central retina. We have now examined RGC in the first time that large numbers of RGC with normally oriented or tangentially growing dendritic trees were displaced bodily into the IPL in such specimens. We subsequently injected horseradish peroxidase (HRP) into the teum to label all with the middle region of the nasra retina in 33 frogs surviving unilateral optic nerve transaction for periods of 3-99 days. Retinocapsular transport deposited HRP in RGC in the main ganglion cell layer in both eyes, but only in one eye, prec. In either project to the tectum, and do not come to project there aboromly during regeneration. In the retinae substrate in optic nerve regeneration, the RGC newly displaced into the IPL, as well as the non-displaced RGC, were labelled with HRP in significant numbers. In a typical retinae section after completing observations on the flat-mount, the thickness of the IPL measured at 219 locations was 14.3-16.9 µm in the affected eyes, and the mean outward dilation of the displaced cell layer was 0.3-1.7% of that distance (M1241). The non-displaced RGC formed a monolayer, as in the normal retina. Supported by PHS grant EY0284 to F.S.

746.15

We have previously shown that many retinal ganglion cells (RGC) in the cat are immunoreactive for N-acetylasparylglutamate (NAAG), a possible neurotransmitter. To determine whether all RGC contain NAAG, and whether all NAAG-positive cells in the ganglion cell layer are ganglion cells, we have attempted to double-label these cells with antisera to HRP and conc. RGC and with AB5, a monoclonal antibody specific for RGC. Three cats were deeply anesthetized and perfused with a mixture of 4% paraformaldehyde and 4% carbodiimide. Large pieces of intact retina were repeatedly frozen and thawed, then incubated for 10 days at 4°C in a mixture of the primary antibodies (anti-NAAG at 1:500; AAB (purified ascitic fluid at 1:10,000), followed by fluorescein-labeled donkey anti-rabbit and rhodamine-labeled goat anti-mouse (each at 1:100) for 2 days. The great majority of labeled cells contained both labels. However, some small cells were labeled for NAAG but not for the AAB antigen. These were more common in peripheral than central retina, and we assume that they are displaced amacrine cells. In addition, a very few cells were AAB positive but did not label for NAAG.

We conclude that some displaced amacrine cells and all but a few RGC contain NAAG. (Supported by NSF grant BNS-8811039 to SBT and NSF grant EY0284 to F.S.)

746.16

Morphological investigations were undertaken in the retina of the frog (Rana esculenta) using either the Golgi or the BHRP (B-subunit of cholera toxin conjugated to horseradish peroxidase) technique. In our sample of 48 cells encountered with the Golgi technique (sagittal sections) we could distinguish 11 main types of retinal ganglion cells. In order to determine their projection sites, the BHRP was injected either into the tectum or the diencephalon. Frog retinas were flat-mounted and processed with DAB. By using a 'depth-differentiating' drawing method, dendritic arbors could be reconstructed and cells correlated to those found with the Golgi technique. 200 cell drawings were analyzed with the morphological cell type (M1-M11) found with the Golgi technique could be confirmed. From these 11 types, 5 could be found after tectum and diencephalon injection, 4 only after tectum injection and one type, M11, only after diencephalon injection. The dendritic tree of the latter type only spread within the fourth sublayer of the inner plexiform layer (IPL) - the most vitreal sublayer. The dendrites did not take off into the IPL and encompassed an area of 960 at 100x a diameter radiating in all directions. The greater soma diameter varied between 14.6 and 16.1 µm. These cell types are probably the 'blue-sensitive' on- units of the N. Belloni (Munz W. R. A.; J. Neurophysiol. 25:699, 1962).

Supported in part by the Deutsche Forschungsgemeinschaft (Gr 276).

746.17

Anti-trypsin hydroxylase stains a population of cells in the Xenopus retina. The soma (12-15 µm diameter) is located in the amacrine cell layer. The cells are unevenly distributed with the highest density of ca. 200 cells/mm² in the caudal lateral part of the retina whereas the frontal medial part contains only a few scattered cells. About 2% of the cells were observed to have processes ascending within the inner nuclear layer. Within the inner plexiform layer (IPL), thick dendrites form a sparse network in the proximal portion, whereas in the distal portion a dense network of fine processes was observed. The distribution of dopaminergic nerve fibers was studied by a factor of 2.3, cell density decreased by 55%, and cell area increased by 234.

Serotonin-like immunoreactivity stains two classes of amacrine cell, the large soma amacrine (L-ser, soma size 12-20 µm) has several primary processes in the IPL. The large soma amacrine (S-ser, soma size 8-10 µm) exhibits only one major process. Processes of both classes ramify diffusely in all sublayers of the IPL. TOH and serotonin-positive processes were used to approximate the TOH, L-ser and S-ser cells are ca. 2:1:5. Additionally two classes of bipolar cells show serotonin-like immuno-reactivity. Supported by EY 03570 to P.W.
476.19

Phenylethylamine N-methyltransferase-containing neurons in the small ear pig retina

During development, both GAD-IR and TOH-IR are barely detectable in the retina at 2d, although staining elsewhere in the brain is intense. At this time, the IPL is just becoming apparent. By 5d, the time of t-titching, retinal staining is distinct and adult-like in distribution. Two features distinguish the development of the two systems. First, TOH-IR is much slower to develop in intensity than GAD-IR. By 15d when GAD-IR is adult-like in intensity, TOH-IR lags far behind. Second, GAD-IR is seen in the optic nerve between 3 and 5d, but disappears by 9d and is not seen in the adult.

Using antibodies against their synthetic enzymes glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TOH), respectively.

476.21
Phenylethylamine N-methyltransferase-containing neurons in the small ear pig retina


The localization of amphetamine-containing neurons in the small ear pig retina has been detected by immunohistochemical labeling of the amphetamine-synthesizing enzyme phenylethylamine N-methyltransferase (PNMT). The PNMT-positive cells are observed in the outer tier of cells of the inner nuclear layer (INL) and their processes are observed as punctate structure in the outer plexiform layer (OPL). The cell soma is around 10-15 um. These amphetamine neurons are suggested to be amacrine cells or horizontal cells. The pattern of distribution has been further confirmed by electron microscopy (EM). This results are similar to PNMT-positive cells in ferret retina (Keyser et al 1987, 7(12):3996-4004), but difference with PNMT-positive cells in rat retina in which cell bodies are found in the INL and ganglion cell layer (GL) and their processes are found in the outer and inner strata of IPL (Hadjiconstantinou et al. 1984, Neuroscience 13:47-551 and D. Park et al 1986, 6(4):1108-1113)

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476.22
IMMUNOCYTOCHEMICAL DISTINCTION BETWEEN INNER AND OUTER HORIZONTAL CELLS IN THE LAMPREY RETINA.


During the development of the retina in the larval sea lamprey, Petromyzon marinus, horizontal cells (HCS) are morphologically distinguishable years before the differentiation of other synaptically-related cell types (Rubinson and Cain, in press). In years before the expression of visual responses (Rubinson, et al. 1977). Two tiers (OHCs and IHCs) are apparent in the adult but, in the larval retina, there is a single layer.

Immunocytochemical studies were undertaken to see if OHCs and IHCs express unique patterns of immunoreactivity (IR). Retinal sections were first transformed, and then the lamprey were reacted to GABA or glutamate anti-tera and processed by an indirect PAP procedure. OHCs were GABA-positive as were their axons which appeared as a few microtubules between OHCs and IHCs. IHCs were distinctly glutamate-positive, as were many small terminal processes in the OPL. Each tier appeared as a unicolor layer, although there was no evidence of any displaced cells of either immunoreactivity. GABA-IR in HCs is expressed in non-mammalian retinas and as a transient developmental feature in mammals. The finding of glutamate-IR in HCs appears to be novel. This distinction in IR between OHCs and IHCs in lamprey is significant for analyses of HC lineage and phenotypic expression. (Supported by NIH RO1 EYO-1662 to SY)

476.24
CATECHOLAMINE AND OTHER CALCIUM-BINDING PROTEINS IN RETINA. J.H. Rogers* (SPON: M. Hanley). Physiological Laboratory, University of Cambridge, Downing St., Cambridge CB2 3BG, U.K.

Catecholamin and calbindin-D28 are two calcium-binding proteins with 60% homology, which are abundant in separate sets of neurons in chick brain and retina (Rogers, J.H., J. Cell Biol. 105, 1343 (1987), and Neuroscience, in press). Their distribution has been investigated by immunohistochemistry in retinae of chick, cat, rat, and salamander (larval Ambystoma tigrinum).

Sections were incubated with rat antisera against a B-galactosidase-calcitonin gene-related protein, with rabbit antisera against calbindin (gift of Dr. E. Lawson), or with rabbit antisera against another calcium-binding protein, parvalbumin (gift of Dr. C. Hellemann). Two-colour immunofluorescence was used.

In all species except rat, there were some common positive for calbindin, and some bipolar cells positive for calbindin or catelinin. Rods were always negative.

In all species, horizontal cells contained large amounts of one or other calcium-binding proteins, with rabbit antisera against calbindin in the cat. In all species, there were many amacrine cells and some ganglion cells positive for one or more of these proteins. In the cat, there were many inclusion cells (or displaced amacrine cells), which appear to be highly symmetric and which stain strongly for calbindin.

This work is supported by the Wellcome Trust.
477.1


Three types of retinogeniculate terminals (R1, R2 and R3) have been identified in the adult hamster (Erzurumlu et al., Brain Res., 461:175-181, 1988), each having a characteristic distribution within the LGNd. Large R1 terminals are located medially, small, clustered R2 terminals form an outer shell lateral and caudal to the R1s and R3 terminals are ubiquitous.

We have studied the maturation of the LGNd and R1 terminals by applying HRP crystals to optic tract axons below LGNd and visualizing the labeled axons and their terminals with HRP-DAB reaction product. Neonatal hamsters on postnatal day 11 were fixed with a use of HRP (PND21). After labeling on PND26 (after segregation of ipsi- and contralaterally projecting axons in LGNd), very few terminals have a definitive, adult-like morphology. At the time of eye opening (PND14), immature LGNd terminals can be differentiated and clusters of terminal and axon present are lateral where R2 terminals will develop. However, it is not until PND21 that morphologically mature features typical of mature R1 and R2 terminals can be delineated. Unilateral eye enucleation results in altered distributions of retinal terminals even when performed up to PND14, albeit the extent of the changes decreases with increasing postnatal age.

Thus retinogeniculate terminals achieve stable, adult-like morphology and distribution several days after the eyes have opened and visually evoked activity has commenced.


477.3


The dorsal lateral geniculate nucleus (LGN) of the ferret affords an opportunity to compare the development of on-center and off-center channels in the brain. Cytarachnionic studies of the ferret LGN (Sanderson, 1974; Linden et al., 1981) revealed sublamination of laminae A and Al into inner and outer leaflets. The outer leaflets of the A laminae were found in a physiological study (Stryker and Zahs, 1983) to contain off-center cells while the inner leaflets contain on-center cells.

In this study, a single pulse of [3H]thymidine was injected into the placenta of ferret fetuses at various developmental ages. After sacrificing these animals as adults, their leaflets were processed with standard autoradiographic techniques and with GABA antisera (Incstar) using immunohistochemical methods. Also, in some of these animals, cytochrome oxidase or ACHE activity was localized histochemically to confirm the boundaries of the sublaminae (Kageyama & Wong-Riley, 1984; Henderson, 1987). The birthdates of neurons in both A laminae of the LGN and the medial interlaminar and perigeniculate nuclei were determined. In addition, GABA-immunoreactive neurons in the outer and inner leaflets of the A laminae were compared in terms of birthdate, density and soma size.

Results of this study indicate that neurogenesis of the ferret LGN begins on or shortly before embryonic day 20 and continues to embryonic day 30. (EYO1338 and EYO3059)

477.5


We have examined the thalamocortical projections in the visual system of the fetal rhesus monkey (Macaca mulatta). Punctate injections of red or green fluorescent latex beads, limited to the cortical plate, were made on either side of the V1/V2 border. Lamination had progressed across only the dorsal half of the lateral geniculate nucleus (LGN) at E95 (gestation: 165 days), whereas by E111 the LGN appeared fully laminated. As in the adult, injections in labeled cells distributed in the parvo- and magnocellular laminae, whereas injections in V2 labeled cells sparsely in the S layers and interlaminar zones. Labeled cells formed topographically ordered columns within the LGN. At E95, shortly after geniculocortical fibers invade the cortical plate (Rakic, 79), a small number of ectopic cells were found outside of the labeled-cell columns, and these cells were scattered within the more ventral (less-mature) layers of the LGNd. By E111 these rare ectopic projections were eliminated. Labeling in the pulvinar also exhibited topographic order and specificity similar to the adult. At least two projection zones were apparent, the one more weakly labeled from V1. These events indicate that in the ferret, these events play only a minor role in the establishment of the specificity and topography of thalamocortical projections to visual cortex. (Supported by RR00169 from NIH)

477.6


Neural connectivity was studied in coculture preparations (2-3 weeks in vitro) of rat fetal lateral geniculate nucleus (LGN) and newborn visual cortex (VC). Morphological studies using Nissl staining and retrograde labeling with HRP or a fluorescent dye (DiI) demonstrated that the VC explant retained almost normal laminar and columnar organization well as neuronal morphology such as pyramidal or stellate cells and that the LGN explant contained multipolar and bipolar cells. Furthermore, the VC—LGN coculture revealed that the LGN axons have characteristic arbors terminated in the granular layer and that the VC axons have characteristic arbors terminated in the infragranular layer of the LGN explant. Electrophysiological studies of extra- and intracellular recordings confirmed that afferent and efferent connections were established between the LGN and VC explants with normal laminar and columnar organization. The results strongly suggest the existence of intrinsic mechanisms controlling the development of appropriate neural connectivity in LGN and VC.

477.7

RETINOGENICULATE FIBERS ARE ESSENTIAL FOR THE PROPER TIMING OF DENDRITIC APPENDAGE ELIMINATION IN THE DORSAL LATERAL GENICULATE NUCLEUS. J. Avery and J. Bruno-Bechtold. Program in Cell Biology and Neuroscience, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

In our ongoing study of the development of neuronal morphology in the dorsal lateral geniculate nucleus (dLGN) of newborn ferrets, we have used a Golgi-Hortega method to compare dendritic development in the dLGN of normal and enucleated ferrets. Following bilateral enucleation at birth, normal neuronal classes develop; however, the timing of filiform appendage development is altered. In the absence of visual input. Normal and bilaterally enucleated ferrets were perfused at ages from birth to maturity. In both groups, filiform dendritic appendages on large, class I neurons appear at the end of the first postnatal month, around the time of eye opening in normal animals. In normal ferrets, all appendages increase in number until P56, before undergoing a significant reduction by P90. In normal adults, most appendages have disappeared, especially on proximal dendritic segments. In bilaterally enucleated ferrets, there is also an exuberant production of dendritic appendages before a decline to normal levels by adulthood, but this decline is delayed. Thus, retinogeniculate fibers are not necessary for the development of dLGN cell classes, but are essential for the proper timing of neuronal maturation (EY05028).

477.8

CHANGES IN THE DISTRIBUTION OF ANTI-FIBRONECTIN IMMUNOREACTIVITY IN THE DORSAL LATERAL GENICULATE NUCLEUS DURING DEVELOPMENT. Judy K. Bruno-Bechtold and Darrell Agee*. Program in Cell Biology and Neuroscience, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Cell layers of the dorsal lateral geniculate nucleus (dLGN) separate from a relatively homogeneous distribution shortly after segregation of the ipsilateral and contralateral retinogeniculate fibers but before eye opening. During this time synaptogenesis in the dLGN is ongoing. In the present study, we determined the distribution of fibronectin (FN) in the dLGN from embryonic day E20 to E45. As cell layers of the dLGN begin to differentiate, FN-immunoreactive cells appear in the dLGN. By P5, most FN-immunoreactive cells are distributed in the dLGN, and a few cells are found in the optic tract or C-layers. During the postnatal period, FN-immunoreactive cells in the dLGN increase in number until P56, before undergoing a significant reduction by P90. P90 is the first time at which the dLGN is fully laminated. Changes in FN-immunoreactive cells with postnatal age are correlated with changes in the distribution of the FN-immunoreactive cells. This change in FN-immunoreactivity is evident in the optic tract as well as medial to the dLGN. During P90, there is labeling in the dLGN, and the FN-immunoreactive cells in the dLGN increase in number until P56, before undergoing a significant reduction by P90. The results of this study suggest that changes in the distribution of FN-immunoreactivity are correlated with changes in FN-immunoreactivity in the dLGN.
747.7


When monocular enucleation in rats on the day of birth (P1) results in an expanded ipsilateral retinotectal projection from the remaining eye in adults, it is believed due to the survival of ipsilaterally projecting retinal ganglion cells which would normally die. If enucleation causes no inhibiton of development of the tectal or the ipsilateral projection to the eyes, responses should appear bilaterally in the colliculi of enucleated neonates. While contralateral responses were first recorded on P12 as in normals, no ipsilateral response could be found up to P15, the oldest age studied thus far. Flask evoked potentials were recorded from the ipsilateral colliculus in a normal adult, demonstrating the sensitivity of the apparatus. The results suggest that after PO enucleation ipsilateral pathway development lags behind contralateral development. The findings correlate with additional studies where P0 enucleation induces transsynaptic transport of tracer in the ipsilateral pathway, but 7 days after contralateral labeling.

747.8


Although the anatomical reorganization produced by monocular enucleation has been the subject of intensive investigation, there is much less information about the behavioral consequences of these early lesions. In young of the northern native cat, a small carnivorous marsupial, received monocular enucleations at ages ranging from 13 to 32 days after birth. The eyes were tested as adults in a visual perimeter. Orienting head and body movements to food stimuli were videotaped and single-frame analysis was used to characterize the movements. Normal controls obtained food by means of a series of discrete head and body movements followed by a reach with the forepaw. The number of such movements depended on both the distance and eccentricity of the target. Monocular enucleates obtained the food using significantly fewer and larger head and body movements. On most trials, these animals employed one slow, continuous movement lasting from trial onset to the end of the reach, regardless of the location of the target. One explanation for these findings is that the expansion of the ipsilateral retinocollicular projection produced changes in the brainstem circuitry subserving orienting movements.

747.9

DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES IN THE NUCLEUS TRACTUS OPTICI OF THE CAT

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Adult cats have a symmetric monocular horizontal optokinetic reflex (OKR), i.e. the eyes follow the stimulus in a direction with equal gain (e.g. stimulus velocity). A prerequisite for this response is the presence of binocular neurons in the nucleus tractus optici (NTO). About 80% of the neurons can be activated by stimulation of both eyes, while 10% show a unidirectional and early visual deprivation as well as strabismus abolish the NTO-binocularity and the symmetry of OKR. NTO-neurons come under the sole control of the contralateral eye and OKN through one eye can only be elicited with temporo-nasal stimulus movement in the visual field. Kittens show asymmetric OKN up to age of about 3-4 months. Therefore, we recorded NTO-neurons in 6 kittens aged 27-47 days to investigate the disproportionate distribution in this structure. Also in these animals 72% of the neurons could be activated by either eye and were direction-selective for ipsi- or contralateral stimulation. The OKR in adult kittens is a most sensitive test in that, in kitten monocular deprivation 60% of the neurons had equally strong influence from both eyes compared to 35% in adult animals. Thus, at the onset of the sensitive period both eyes are connected to the NTO-neurons but the ipsilateral eye's influence is much weaker. Balanced binocularity develops rather late during the sensitive period (probably only after three months). This explains why OKN is asymmetric in kittens. Visual deprivation or strabismus must even disrupt the feed forward influence of the ipsilateral eye on NTO-neurons.

747.11

PROTEIN COMPONENTS OF THE RETINOTETICAL ACTIVITY DEPENDENT MAPPING MECHANISM. P. Sporn* and M. Constantin-Paton. Biology Department, Yale University, New Haven, CT 06511.

The NMDA receptor appears to be a component of the activity dependent mechanism which organizes the retinotopic map within the frog tectum. Chronic treatment with NMDA antagonists, APV and NMDA receptor specific agent (Clime and Constantin-Paton, 1988, Neurouns. Abstr. 14(4)). In addition APV causes degeneration of specific optic fibers in 3-day-old animal and treatment with NMDA antagoist prevents the degeneration of thalamic fibers in adult animal. The results suggest that NMDA receptor activation triggers a cascade of events leading to the ordering of the retinotopic map within the tectal or of the retinal projection cells synapses on the same post-synaptic membrane. In order to identify other molecular components of this activity-dependent mechanism we are using two-dimensional gel protein analysis to analyze differences in the protein composition of normal and striped tecta, and molecular changes induced by chronic APV and NMDA treatment. Preliminary results indicate a number of changes in both cases, including a decrease in an acidic protein of approximately 110 kDa. APV versus NMDA treated animals and a decrease in an acidic protein of approximately 30 kD in the doubly versus singly innervated tecta. We have investigated the role of serotonin and that the changes in the central serotonergic system occur during aging in the cockroach. We have investigated the changes in serotonergic immunoreactivity in the optotectum which include a decrease in the density of the serotonin-containing fibers. This projection is thought to be involved in sensory processing and has been shown to be affected in monocular enucleation. The serotoninergic projection from the nucleus raphe dorsalis produces changes in the contralateral thalamic projection in the cat. The serotoninergic projection in the cat is thought to be involved in sensory processing and has been shown to be affected in monocular enucleation. The serotoninergic projection from the nucleus raphe dorsalis produces changes in the contralateral thalamic projection in the cat. The serotoninergic projection in the cat is thought to be involved in sensory processing and has been shown to be affected in monocular enucleation.
Peripheral retinal axons in chicks initially target along the two main axes, medial-lateral (M) and rostral-caudal (R), of their target, the contralateral optic tectum. They then spread into the tectum from other parts of the chick retina behaviorally. Small areas of retina (> 0.1%) were labeled with DiI in embryos and adults, and filled, and whole mounts of retina and tectum made. In the mature projection, labeled peripheral nasal axons project to a terminal zone (TZ) at the caudal tectal border, but are distributed across most of the M-L tectal axis more rostrally, indicating that they made major changes in position as they grew across the tectum. Developing nasal axons occasionally make short, transient, and short-lived rostral tectal connections. After maturation, axons labeled from peripheral dorsal retina at its naso-temporal midline project to a TZ in far lateral tectum, near its R-C midline. Earlier injections labeled only a posterior compartment at the edge, but spread uniformly over the lateral third of tectum. Some axons enter tectum far medially, a few are found in between. As the axons grow caudally, most grow past their TZ, some continuing to the caudal border. Branches and arbor form rostral to the TZ. After maturation, axon trajectories indicate that many make course corrections to enter the TZ, but most surviving axons are found within 500 μm of the TZ. These axons enter tectum far medially are not present. Injections in peripheral ventral retina label a mirror-image pattern to that seen for dorsal injections. After maturation, injections in central retina label a TZ in central tectum, but again many axons make trajectory changes along the M-L and R-C axes to achieve their TZ. At earlier stages, many central axons also overshoot their TZ, but do not grow distally into caudal tectum; typically branches and arbor form along their length. Thus, central retinal axons as well as late arriving axons from peripheral retina, initially mistarget along the M-L and R-C tectal axes, but often correct these errors, attain their appropriate TZ, and survive.

Activity is either blocked with tetrodotoxin (TTX) or synchronized with a strobe light (Hz) in regenerating projections from 2 to 6 weeks postcrush. HRP ipsilateral injections were made into the retina, and tracers were drawn by electron microscopy from the axon terminals. Many axons originated in the same three calibers of axons (fine, medium, and coarse), were associated with the same three sizes of arbors (small-12μm, medium-21μm, and large-27μm) but had about 18% of the large-27μm and about 21 branches and terminated at the same depths. However, they were on average 10% larger in Hz extent. TTX and strobe regenerated arbor at 8 weeks were 71% and 10% larger, respectively, than the controls. However, large branches did not form as approximately the same number of branch endings. In fact, all three classes were larger and abnormal in appearance. Thus, the one significant effect of manipulating activity was to enlarge the spatial extent of the arbor, as expected since these treatments prevent the sharpening of the retinotopic map as assessed electrophysiologically. Nonregenerating projections undergoing similar TTX and strobe treatments were essentially normal but showed slight enlargement (20 and 38%, respectively). Since Schmidt et al. (1988 J.Comp.Neuro 229:545-591) previously showed that regenerating axons initially make widespread branches and later retract many of those branches, the present findings support the idea that blocking activity or synchronizing activity interferes with the elimination of some of the erratic branches and with the focussing of branches into a single cluster. (Supported by NIH grant EY-03736).

AFFERENT TARGET DISORDERS IN THE MAMMALIAN RETINOCECTAL SYSTEM ARE BALANCED BY CHANGES IN RETINAL AXON ARBORIZATION AND PROJECTION PATTERNS. B.L. Finlay and S.I. Pallas. Dept. of Psychol., Cornell University, Ithaca, NY 14853. We present data suggesting a lack of change in convergence between retinal cells and single tectal cells. The present study was designed to determine whether this is accomplished of Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

ABSORPTION ACTIVITY IN GOLDISH OPTIC NERVE IS ASSOCIATED WITH THE POLYSYNAPTIC Field POTENTIAL IN OPTIC TECTUM. W. M. King and J. T. Schmidt. Dept. of Psychology, SUNY at Albany, NY 12222.

In an in vivo preparation of goldfish optic tectum and full length optic nerve, shocking the nerve leads to an orthodromic compound action potential (CAP) in the nerve and an antidromic field potential (FP) in thalamus, followed by a polysynaptic FP 12-16msec later. Associated with the polysynaptic FP is a rebound CAP recorded from the end of the nerve at a latency of 20-25msec. The stump of tectal origin was indicated by its long latency and its disappearance after cutting the optic tract central to the stimulating electrodes. In addition, manipulations that block the polysynaptic FP also block the ricochet or FP. The long latency polysynaptic FP was blocked by 1.5 μM carbachol, atropinum, or curare, by high [Mg] or by some other means.


Activity is either blocked with tetrodotoxin (TTX) or synchronized with a strobe light (Hz) in regenerating projections from 2 to 6 weeks postcrush. HRP ipsilateral injections were made into the retina, and tracers were drawn by electron microscopy from the axon terminals. Many axons originated in the same three calibers of axons (fine, medium, and coarse), were associated with the same three sizes of arbors (small-12μm, medium-21μm, and large-27μm) but had about 18% of the large-27μm and about 21 branches and terminated at the same depths. However, they were on average 10% larger in Hz extent. TTX and strobe regenerated arbor at 8 weeks were 71% and 10% larger, respectively, than the controls. However, large branches did not form as approximately the same number of branch endings. In fact, all three classes were larger and abnormal in appearance. Thus, the one significant effect of manipulating activity was to enlarge the spatial extent of the arbor, as expected since these treatments prevent the sharpening of the retinotopic map as assessed electrophysiologically. Nonregenerating projections undergoing similar TTX and strobe treatments were essentially normal but showed slight enlargement (20 and 38%, respectively). Since Schmidt et al. (1988 J.Comp.Neuro 229:545-591) previously showed that regenerating axons initially make widespread branches and later retract many of those branches, the present findings support the idea that blocking activity or synchronizing activity interferes with the elimination of some of the erratic branches and with the focussing of branches into a single cluster. (Supported by NIH grant EY-03736).


We have tested whether chronic application of N-methyl-D-aspartate (NMDA) can restore the ability of isthmal axons to shift their connections in 8-month postmetamorphic frogs. The left eye was rotated 90° clockwise. Slabs of slow-release elvan polymers impregnated with the drug were inserted into the right eye and left eye sockets so that the drug would diffuse from the right tectal lobe in half of the frogs; the other eye-rouged frogs served as controls. After a survival period of 3 months, the contralateral and ipsilateral maps to the right tectum were recorded. In contrast to the normal frogs, the two maps were aligned with respect to each other, and the ipsilateral fields often were weak. In contrast, in the NMDA-treated frogs, most ipsilateral receptive fields were in register with the contralateral map and were aligned with the same size.

We thus conclude that plasticity in this system can be restored by chronic application of NMDA. This result implies that the duration of ipsilateral plasticity during development is normally limited by some change in the NMDA receptor or by processes triggered by activation of the receptor.

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The condition of Xeapous frogs receives input from both eyes. The contralateral eye's projection reaches the tectum directly, via the optic nerve, and the ipsilateral eye's projection reaches the tectum indirectly, via the nucleus isthmi. The ipsilateral map shows great plasticity during development and is modified by a variety of lesions. We tested whether altering the rotation of either eye, but this plasticity normally ends by about 3 months after metamorphosis. The process by which the ipsilateral map comes into register with the contralateral map involves many parallel and interacting activity, and we have shown that normal function of the NMDA-type glutamate receptor is essential to this matching process.

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Supported by USPHS Grant EY-03470 to S.B.U.

TEMPORAL PROFILE OF THE "CRITICAL PERIOD" FOR INTERTECTORAL PLASTICITY IN XENOPUS LAEVIS: RELATION TO NORMAL DEVELOPMENTAL DEMAND AND EXTENSION BY DARK-BEARING.

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(SFON: Brain Research Association) National Institute for Medical Research London NW 1 1AA UK

A common system of intertectal connections mediates binocular visual integration in Xenopus. During development, this system displays plastic adjustments in response to marked changes in eye alignment which commence at metamorphosis and continue at a reducing rate for some months thereafter. This normal plasticity utilizes visual experience. The plastic capacity of the system may be revealed by challenging it to adjust to surgical eye rotation. The system shows a high plastic capacity (ability to respond to large eye rotations) at metamorphosis and a progressive reduction in capacity over a 3-month period afterwards. The profile of this capacity mirrors temporal features of the normal changes in eye alignment. Visual experience is not only utilized to effect intertectoral plasticity but also contributes to its age-related reduction. Animals deprived of vision during the normal critical period, and indeed for an extended time after it, show the high plastic capacity normally only seen in metamorphosing animals.

EPILEPSY: EXCITATORY AMINO ACIDS

478.1

ROLE OF NMDA AND NON-NMDA RECEPTORS IN Picrotoxin-INDUCED EPILEPTIFORM ACTIVITY IN RAT NEOCORTEX. W. L. Le* and J.J. Habelitz

(SFON: P. Kellaway) Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294

D-2-amino-5-phosphononic acid (D-APV) and 6-cyano-2,3-dihydroxy-7-nitroquinazoline (CNQX) are antagonists of NMDA and non-NMDA excitatory amino acid receptors, respectively. The involvement of these two classes of receptors in generation of picrotoxin-induced interictal discharges in adult rat neocortex was examined. D-APV (20 µM) reduced the amplitude of the PDS by 15±3% (n=8). PDS duration (measured at 50% of peak amplitude) was reduced by 36±8% (n=7). Complete suppression of PDS was never achieved with D-APV. CNQX (5 µM) had no effect on the peak amplitude of the PDS in 83% (n-6) of the cells tested, but delayed the offset of the PDS and reduced its duration by 56±1% (n=4). In four cells, CNQX was able to abolish evoked epileptiform activity. Control PDSs were shorter in these neurons than in cells where PDSs were not blocked by CNQX (42±9 vs 190±20 ms). PDSs were always abolished when D-APV and CNQX were applied together, suggesting that an NMDA-mediated component is unmasked in the presence of CNQX. These results indicate that both NMDA and non-NMDA receptors are activated during PDSs in the rat neocortex. NMDA receptors contribute to the PDS but are not necessary for its generation. Non-NMDA receptor antagonists are capable of completely blocking epileptiform activity. Supported by NS18145 and NS22373.

478.2

KINDLING LIKE INDUCTION OF ELECTROGRAPHIC SEIZURES IN VITRO INCREASES ELECTIC ACTION POTENTIAL GENERATION THROUGH NMDA RECEPTOR-DEPENDENT MECHANISMS. W. A. Wilson and S. F. DeCarolis

(Dep. of Pharmacology and Medicine, Duke Univ. and V.A. Medical Centers, Durham, N.C.)

We have previously described an in vitro model of kindling-like epileptogenesis in which electrographic seizures (EGSs) are induced in rat hippocampal slices through N-methyl-D-aspartate (NMDA) receptor-dependent mechanisms. EGS induction was accompanied by a marked increase in the occurrence of "baseline spikes," action potentials which arise sharply from the baseline membrane potential, and appear to be generated from an "ectopic" site distant from the soma. Here we report that this increase is also NMDA receptor-dependent.

EGSs were induced in rat hippocampal slices with repeated stimulus trains. In parallel, CA3 pyramidal cells displayed a marked increase in the frequency of baseline spike firing (Harrington, ABSEI, 1989). In an additional 12 experiments, cells were continuously recorded in the presence of the NMDA receptor antagonist D-APV (100 µM). APV prevented EGS induction in all cases, and the increase in baseline spiking in 9. In one case, APV allowed a mild enhancement of afterdischarges; in parallel, baseline spikes began to fire at a low frequency (0.4/min.). Upon washout of APV, afterdischarges developed into full EGSs, and the frequency of baseline spikes increased markedly (30/min.). Thus, EGS induction and increased ectopic action potential generation were both dependent on NMDA receptor activation.

478.3

Epileptiform bursts induced by 4-aminopyridine (4AP) in the rat hippocampus: possible mechanisms. P. Perreault and M. A. Avoli

McGill University, Quebec, Canada. H3A-2B4

Disinhibition and NMDA receptor activation have been considered as important factors in epileptogenesis. Here we have used conventional intra- and extracellular recordings to study in the CA1 subfield of rat hippocampal slices the physiological bases of 4AP-induced activity. Intracellular recordings with QX-314-filled microelectrodes showed that the spontaneous field bursts (SFBs) were associated with 22-38 mV giant EPSPs. Both SFBs and giant EPSPs were prevented in the presence of 4AP and APV, but were blocked in a dose related way by CNQX (3-10 µM). Under these conditions, BMI insensitive, spontaneous and evoked IPSPs were seen. There was a strong correpondence between CNQX and CNQX and CNQX was similar (IC50 1.5xM). We conclude that SFBs induced by 4AP do not require disinhibition or NMDA receptor involvement.

478.4

SUSTAINED ANXIOLYSIS ACTION OF 3-(2-CARBOXY-PIPERAZIN-4-YL)-1-PHOSPHONATE (CPP-ENE) FOLLOWING I.V. OR ORAL ADMINISTRATION IN YAKA PAPIONS. S. Patel* and B.S. Meldum


CPP-ene is an unsaturated analogue of 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) that has been shown to be more potent than CPP as an NMDA preferring receptor antagonist. It is orally active in the rat electroshock test (Herrling et al., this meeting). CPP-ene was administered either i.v. (76 mg/kg) or orally (8-64 mg/kg) to baboons, Papio papio, with photosensitive epilepsy. CPP-ene, 8 mg/kg, i.v. provoked subclinical EGSs that showed a marked suppression of phytotonically-induced myoclonic responses that lasts 24-48h. Oral administration of CPP-ene, 32-64 mg/kg, lead to complete suppression of phytotonically-induced muscular responses that lasts 24-48h. Oral administration of CPP-ene after i.v. or oral administration. Neurological side effects were seen after 24h and were not observed in baboons. These properties indicate a potential for clinical use in epilepsy.

The intracranial stimulation in the central nervous system are mediated through GABA receptors. Activation of presynaptic GABA autoreceptors located on GABAergic neurons can result in an inhibition of GABA release, leading to a net disinhibition of responses. We have examined the effects of D-APV on the inhibition of evoked responses produced by (-) baclofen in the dentate gyrus of the rat.

Rat hippocampal slices were maintained in an interface recording chamber. Extracellular population spikes were recorded in the granule cell layer of the dentate gyrus in response to medial perforant path stimulation. A 15 min bath application of baclofen lasted through a 30 min wash period. At a concentration of 5µM, baclofen also induced epileptiform activity as indicated by the occurrence of multiple (2-4) population spikes, and a loss of paired pulse inhibition at interpulse intervals of 20-30 msec. This activity was also specific to the dentate gyrus, as population spikes recorded in field CA3 were reversibly depressed by baclofen (mean 50% of control). A 15 min bath application of APV (10µM) produced a depression of the evoked response (mean 71%). When APV was applied for 15 min before, during, and after baclofen (5µM), the occurrence of multiple population spikes was reduced (1-2). In addition, APV could reverse baclofen-induced epileptiform activity. We hypothesize that baclofen acts primarily presynaptically in the dentate gyrus to inhibit GABAergic and, thus, abolish this activity. These findings further support the potential usefulness of NMDA receptor antagonists as antiepileptic agents. (Sup. by NIH grant NS23865)


Glycine has been found to be a requirement for NMDA receptor activation (Kleckner, N.W. and Dingledine, R. Science 241:835-837, 1988). The NMDA receptor antagonist, HA-966 appears to compete with glycine at the glycine modulatory site of the NMDA receptor complex (Lodge, D. In: Cavalheiro, E.A. et al. (eds) Frontiers in Excitatory Amino Acid Research, in press). Another agent, kynurenic acid, which is an excitatory amino acid receptor and strychnine-insensitive glycine receptor antagonist, has been shown to antagonize amygdalar kindling in rats (Denenhofer, J.L. and Cain, D.P. Soc. Neurosci. Abstr 13:761, 1987; Thompson, J.L. et al., Epilepsy Res 2:302-308, 1988). To evaluate the effect of HA-966 on kindled seizures, rats were injected with bipolar electrodes in the basolateral amygdala (AP-22.2 mm, Lat-4 mm, Vent-8.5 mm) and a cannula (AP-1.0 mm, Lat-1.6 mm, V-3.5 mm) in the lateral ventricle. Beginning 7 days post-surgery, subjects received daily stimulation (10 sec train, 1 msec biphasic pulses, 60 Hz, 200 microamps base-to-peak) until a criterion of five cumulative stage 5 seizures was obtained. Following a 30 minute pretreatment with HA-966 (100 or 200 microgm in saline given to fully-kindled rats, i.c.v.) failed to significantly alter seizure score (mean 71%). When APV was applied for 15 min before, during, and after baclofen (5µM), the occurrence of multiple population spikes was reduced (1-2). In addition, APV could reverse baclofen-induced epileptiform activity. We hypothesize that baclofen acts primarily presynaptically in the dentate gyrus to inhibit GABAergic and, thus, abolish this activity. These findings further support the potential usefulness of NMDA receptor antagonists as antiepileptic agents. (Sup. by NIH grant NS23865)

748.7 DEXTROXORPHIN INHIBITION OF PENCILLIN-INDUCED EPILEPTIFORM DISCHARGES IN RAT HIPPOCAMPAL SLICE. J. Arvanpur*, A.E. Cohn, C.U. Feoklis, R.R. Fishman, Department of Neurology and Neurosurgery, The Johns Hopkins University School of Medicine, and Department of Pharmacology, University of Maryland School of Pharmacy, Baltimore, MD 21205.

We studied the anticonvulsant effect of dextrophan in the rat hippocampal slice. Dextrophan is a metabolite of dextromethorphan with NMDA antagonist properties against penicillin-induced epileptiform bursting. Extracellular and intracellular recordings were performed in pyramidal neurons from region CA1 of the rat hippocampal slice. In control perfusate a single field population spike was evoked by afferent electrical stimulation. Stimulation after addition of 3.4 mM penicillin (PCN) to the slice perfusate produced 5 or 6 population spikes, reflecting repetitive synchronous firing of pyramidal cells. DX doses of 100 µM were found to increase the mean number of population spikes to 1.5x 2.0x that of control, decreased the mean amplitude of the 2nd-6th spikes to 0.4-0.6x of control and usually abolished the last three population spikes (p<0.05). Depression of the evoked field was maximal at 40 minutes after start of DX perfusion, and rapidly reversed with 90 minutes washout. The dose-response curve of DX was biphasic. Perfusion with 10 µM DX increased the mean number of population spikes to 128.7 x 176.7% of control (n=10, p<0.05). This enhancement was more pronounced with the 2nd-6th population spikes.

Intracellular recordings (n=6) showed that DX 100 µM did not affect mean cell RMP, input resistance, or response to direct depolarizing current. In contrast, 100 µM DX decreased the amplitude of the electrically-evoked, penicillin-induced paroxysmal depolarization shift (PDS) to 60 ± 8% of control (p<0.05). DXC induces slice epileptiform activity by suppressing GABAergic inhibitory mechanisms. The ability of DX to suppress this non-NMDA mediated epileptiform activity confirms its potential usefulness in the treatment of seizures. Caution is warranted, however, in view of a possible enhancement of epileptiform activity at low doses.

ANTICONVULSANT ACTIVITIES OF 1-PHENYLCYCLOHEXYLAMINE ANALOGS. S. Yamaguchi, A. Turkas* and M. A. Rogawski, Medical Neurology Branch, NINDS and Section on Drug Design and Synthesis, NIDDK, NIH, Bethesda, MD 20892.

We previously reported that 1-phenylethylamine (PCA), like its analog the dissociative anesthetic phencyclidine (PCP), is a potent anticonvulsant in the maximal electroshock (MES) test. However, in contrast to PCP, PCA fails to cause motor impairment at anticonvulsant doses (M.A. Rogawski et al., J. Pharm. Exp. Ther. 249, in press). In the present study, we determined the activities of 38 PCA analogs in the mouse MES seizure and horizontal screen motor toxicity tests. Our analogs had the PCA nucleus modified in the following ways: (i) methyl, methoxy, fluoro, trifluoro, or chloro groups, (ii) 4-naphthyl substitutions on the phenyl ring, (iii) modifications in the alkylation ring size. In addition, we examined the stereoselectivity of PCA and its analogs. The ED50 values for protection against MES seizures of the compounds ranged from 4.6 to 40 mg/kg whereas the ED50 values in the toxicity test ranged from 1.6 to 78 mg/kg. There was a wide variation in the therapeutic index (Ti=ED50/ED50 of the compounds (3.4-3.5; PCA, 2.3). In fact, even those compounds with similar ED50 values often had considerably lower Ti values. The compounds with the highest Ti values were cis-4-methyl-PCA and phenyl-cyclopropylamine. We conclude that certain analogs of PCA have a substantially enhanced therapeutic index. These analogs may provide a basis for the development of PPO-related anticonvulsants that share PCA’s potent antiseizure activity but have less toxicity.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
KETAMINE PROTECTS AGAINST BRAIN DAMAGE FROM PILOCARPINE SEIZURES IN ENTORHINAL-KINDLED RATS. D.G. Fujikawa, C.G. Hanson, T. S. Match, and H.L. Langley. V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

The non-competitive N-methyl-D-aspartate (NMDA) antagonist ketamine may be neuroprotective in global ischemia. We studied its effect on the brain damage produced by pilocarpine (PC) seizures in kindled SD rats. After entorhinal (ER) kindling and a rest period of at least 2 weeks, the rats received ketamine, 100 mg/kg i.p., 15 min prior to PC. In 5 rats, 200 mg/kg, i.p., and in 3 rats, 0.2 mg/kg, i.p.хожа, were given 30 min before the PC seizure. The severity of neuronal necrosis was evaluated on a 0 to 3+ scale.

Rats without ketamine showed neuronal damage of 2+ to 3+ in piriform and ER cortex, 1-3+ in amygdala and CA1-4 of hippocampus, 1-2+ in dentate gyrus and thalamus, and 1+ in cerebral cortex and septal nuclei (m3). However, following PC, rats with ketamine (m3) had no neuronal necrosis except for 1 rat which showed unilateral 2+ damage to dorsal CA1-3 neurons in 7/13 sections (6/13 were normal). Besides showing that ketamine reduces seizure-induced brain damage, these results point to a dissociation between its neuroprotective and anticonvulsant effects and suggest that the damage may be NMDA-receptor-mediated.

EFFECT OF CA²⁺-CHANNEL BLOCKERS ON N-METHYL-D-ASPARTATE (NMDA)- AND QUISQUALIC ACID (OA)-INDUCED SEIZURES. H.S. White, M.A. Singh, R. Childers, and J. Sackellares. V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Pharmacol., & Toxicol., Univ. of Utah, Salt Lake City, UT 84112 and 1Marlon Labs, Kansas City, MO 64137.

Intracerebroventricular (icv) administration of the glutamate agonists NMDA or OA induces a seizure activity culminating with forelimb tonic extension (FTE). The Ca²⁺-channel antagonists flunitrazepam, diltiazem, and its analogs were used to evaluate their ability to block FTE induced by icvNMDA and icvOA. Groups of 5 to 11 CF1 mice were pretreated (i.p.) for 30 min with increasing doses of the antagonist which were challenged with the convulsive dose 97 of NMDA (3.0 µg/5 µl, icv) or OA (42 µg/5 µl, icv). Animals not displaying FTE within 30 min were considered protected. The most potent against NMDA-induced FTE followed by diltiazem and TA3090 (IC50's: 3.57, 16.6 and 37.2 ±µg/kg, respectively). All three agents were effective against OA-induced FTE (ED50's: 9.6, 67.4, and 122 ±µg/kg for Flunitrazepam, TA3090 and diltiazem); however, at doses of diltiazem causing complete protection, the rotorod test was observed. These results suggest that agents which block Ca²⁺ influx also block the excitatory effects of two glutamate antagonists. Development of Ca²⁺ antagonists for the treatment of seizure disorders. (Supported by a grant from Marion Laboratories and NIH Contract NO1-NS-4-2361.)

The effect of sigma receptor agonists on the hypothalamic-pituitary-adrenal (HPA) axis was evaluated in rats. Adult male rats were injected intraperitoneally and sacrificed after 30 min. Trunk blood was obtained, the plasma separated, and ACTH levels determined using RIA techniques. (+)-Pentazocine, a sigma agonist, was used to potentiate ACTH release. The response was dose-dependent and not reversed by naloxone (6 min pretreatment). Thus this effect did not appear to be mediated via opioid receptors. Similar results were seen using (+)-SKF 10,047 (NAAM): circulating ACTH levels increased significantly in a dose-dependent manner and were not reversed by naloxone. Moreover, the increase in ACTH levels was mediated within the central nervous system. This implies that sigma receptors modulate the HPA axis through a central mechanism.
479.5

This study was undertaken to determine if protein kinase C (PKC) by β-stimulate 1,2-activate (PMA) would alter the rate or magnitude of δ-opioid receptor downregulation in NG108-15 (NG) cells. Incubating NG cells with 1 nM etorphine alone produced delayed down-regulation. However, NG cells cultured with 1 nM etorphine and 30 nM PMA displayed evidence of δ-receptor downregulation greater than that obtained with etorphine alone. Culturing NG cells with 1 nM etorphine alone produced delayed down-regulation. After 24 or 48 hr of treatment, a time period sufficient for PMA to downregulate PKC, the reductions of binding by etorphine with or without PMA were the same, approximately 20-30%. Scatchard analysis revealed that the PMA-induced decrease in binding was due to a reduction in the Bmax value and not a change in affinity. The PMA enhancement of downregulation was blocked by naloxone or by substituting an inactive analog, 4α-phorbol, for PMA. These results suggest that PKC activated PKC can enhance opioid agonist-induced downregulation before the enzyme itself is downregulated.

479.6
CHARACTERIZATION OF THE EFFECT OF STEROID HORMONES ON STRIATAL D2-DOPAMINE RECEPTORS. D. Levesque and D. Di Paolo. Dept. of Molecular Endocrinology, University of Medicine Centre, CHUL, Quebec G1V 4G2 and School of Pharmacy, Laval University, Quebec, Canada.

We have shown that estradiol (E2) and progesterone (P) at physiological doses, acutely increase dopamine (DA) and its metabolite levels. A dose of 100 ng, s.c. also induces a conversion of the high to low affinity state of the D2 dopamine receptor 30 min after the steroid injection. This report investigates the in vitro effect of these hormones on D2 dopamine receptor. We observe that the dose of estradiol do not affect D2 DA receptors as measured by apomorphine competition of [3H]labeled (D2) agonist binding to the high to low affinity state of the D2 DA receptors. When E2 (1 nM) is included, this E2-dependent shift of affinities is prevented. However, addition of P is, which is known to alter agonist binding properties of D2 DA receptors, abolishes this E2 effect. Although P shares common characteristics with E2, on DA release activity, this steroid hormone does not alter agonist properties of D2 DA receptors neither with an in vivo injection of P (100 ng) nor when added (100 nM) into in vitro competition experiments. These results suggest that the rapid increase of striatal DA release produced by E2 could be mediated by an interaction with a δ protein, which in turn affects the agonist states of the D2 DA autoreceptors to ultimately induce DA release. P does not interact with this protein and seems to have an indirect action on DA release. Supported by the MRC of Canada.

479.7

In male Sprague-Dawley rats (n=11) given a unilateral 6-OHDA lesion in the SN, mRNA for D2-receptor was detected by complementary (c)DNA and D2 receptor binding was decreased in the SN and VTA and a concomitant increase in D2 receptor mRNA coding for the putative sixth and seventh intracellular loop of the receptor was observed. autoradiography and in situ hybridization experiments in the caudate putamen, nucleus accumbens, limbic cortex, and olfactory tubercle, although specific hybridization was identified in all traditional dopamine projection areas. In addition, high levels of D2 receptor mRNA were visualized in the substantia nigra, ventral tegmental area, zona incerta, presumably reflecting autoreceptor synthesis. In our ongoing characterization of D2 receptor mRNA in brain, we have examined changes in this mRNA following treatment with dopamine agonists and antagonists. Male Sprague-Dawley rats were treated with chronic haloperidol (14 days, 2 mg/kg/day) or apomorphine (7 days, 5 mg/kg/12 hrs). Following these treatments, brains were removed, frozen, and sectioned. 15 um sections were examined by in situ hybridization using 35a-labelled riboprobes complementary to mRNA coding for the putative sixth and seventh transmembrane domains and the G-protein-associated third cytoplasmic loop of this receptor. Results of these analyses in both traditional dopamine projection fields and in areas associated with dopamine-containing cell bodies will be presented. This work supported by Grants DA02265, MH442251, MH45614, and funds from The Thelphep Raphael Fund and The Lucille P. Markey Charitable Trust.

479.8
LOCALIZATION AND REGULATION OF BRAIN D2-DOPAMINE RECEPTOR mRNA. H.J. Meador-Woodruff, A. Mansour, J.K. Bunow*, H.H.M. Von Toil*, O. Civelli* and S.I. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48106-0720; Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201.

The rat D2 receptor has recently been cloned (Bunzow et al., Nature 336:783-787, 1989) and its distribution has been described in the rat brain. These studies have provided a framework for examining the regulation of this receptor at both the level of its mRNA and binding site. Male Sprague-Dawley rats (N=11) were given a unilateral 6-hydroxydopamine (6-OHDA) lesion in the mesencephalic bundle (8 µg/µl, 15 µg/kg DMI pretreatment) and sacrificed 16 days later. Such lesions produce a dopamine deficiency in the substantia nigra (SN) and ventral tegmental area (VTA) resulting in denervation of the nigrostriatal dopamimergic projection areas. In addition, high levels of D2 receptor mRNA were visualized in the substantia nigra, ventral tegmental area, zona incerta, presumably reflecting autoreceptor synthesis. In our ongoing characterization of D2 receptor mRNA in brain, we have examined changes in this mRNA following treatment with dopamine agonists and antagonists. Male Sprague-Dawley rats were treated with chronic haloperidol (14 days, 2 mg/kg/day) or apomorphine (7 days, 5 mg/kg/12 hrs). Following these treatments, brains were removed, frozen, and sectioned. 15 um sections were examined by in situ hybridization using 35a-labelled riboprobes complementary to mRNA coding for the putative sixth and seventh transmembrane domains and the G-protein-associated third cytoplasmic loop of this receptor. Results of these analyses in both traditional dopamine projection fields and in areas associated with dopamine-containing cell bodies will be presented. This work supported by Grants DA02265, MH442251, MH45614, and funds from The Thelphep Raphael Fund and The Lucille P. Markey Charitable Trust.

479.9

The chronic administration of neuroleptics produces an up-regulation of D3 receptors which may contribute to the adverse motor side effects of these compounds. Chronic treatment with clozapine, an atypical antipsychotic with a low incidence of dyskinetic reactions, is effective in reducing the chronic administration of the SH2 antagonist ritanserin (RIT) affects the D2 receptor up-regulation produced by haloperidol (HAL). Rats were treated for 21 days with RIT (5 mg/kg, ip), HAL (1 mg/kg, ip), both RIT and HAL or vehicle of and killed 3 days following the last dose. Brain sections were prepared for D2 and SH2 receptor autoradiography with [3H]spiperone. Striatal D2 receptors were significantly increased in HAL-treated rats, with the greatest up-regulation in the ventrolateral striatum. Chronic RIT alone decreased the density of cortical SH2 receptors but had no effect on D2 receptors. In a RIT/HAL treatment, RIT receptors were up-regulated together with a significant decrease in the number of RIT receptors suggesting that the chronic administration of SH2 receptors produced by chronic treatment with SH2 antagonists does not affect neuroleptic-induced D2 receptor up-regulation.

479.10
NEONATAL 6-OHDA DENERVATION ELEVATES DOPAMINE D3 BUT NOT D2 RECEPTORS IN ADULT RAT NEOSTRIATUM. K.M. Dewar, J.J. Sophononjan, J. Bruno, L. Descartes and T. Reader. CNR, Dep. de physiologie, Univ. de Montréal, Montréal, (Qué.) Canada and Department of Psychology, Ohio State University, Columbus, Ohio, U.S.A.

The binding properties of [3H]SCH23390 and [3H]raclopride were investigated to evaluate D3 and D2 dopamine (DA) receptors in homogenates of rostral (rCAU) and caudal (cCAU) neostriatum. D2 receptors were significantly increased in the rCAU at 1 and 3 months. In addition, 5-HT and 5-HIAA concentrations were considerably augmented in the rCAU at 1 and 3 months. There were no changes in D2 receptor binding in the rCAU or cCAU at either time period. In contrast, the density of D2 receptors was increased in both the rCAU and cCAU at both time periods. These results indicate that, in the neostriatum, D2 and not D3 receptors are modified by a neonatal DA denervation. Furthermore, since the up-regulation of [3H]raclopride sites is restricted to the rCAU which also exhibits a 5-HT hyperinnervation, these results suggest that this down-regulation of D2 receptors but not D3 receptors is located on the 5-HT fibers and/or regulated by the 5-HT system.

[Supported by the MRC (Canada) and the FRSQ (Québec).]

Binding of [3H]-QNB to intact cells was used to assess the effects of phorbol ester on down-regulation of muscarinic receptors (MR) in 1321N1 human astrocytoma cells. The agonist carbachol induced down-regulation of MR to about 20% of control levels, with a half-time of about 2 hrs. Phorbol 12-myristate, 13-acetate (PMA) alone did not induce MR down-regulation. Inclusion of PMA during carbachol-induced down-regulation inhibited both the rate and maximal extent of receptor down-regulation. The protein kinase C (PKC) activator mezerein, phorbol dibutyrate, and B-phorbol diketacetate also inhibited MR down-regulation, whereas the inactive analog a-phorbol diketacetate was ineffective. The PKC inhibitor staurosporine did not inhibit carbachol-induced down-regulation but did prevent the inhibitory effect of PMA. The effect of PMA on down-regulation was observed in the presence of cycloheximide, suggesting that activation of PKC inhibits loss of MR rather than stimulating MR synthesis. (Supported by GM34500 and HL01993).

MUSCARINIC AND NICOTINIC RECEPTORS AND THEIR mRNA ARE REGULATED DIFFERENTLY IN CULTURED SYMPATHETIC NEURONS. K.R. Smith, Y. Kong, and J.A. Kessler. Departments of Neurology and Neurosciences, Albert Einstein College of Medicine, Bronx, New York, 10461.

Sympathetic neurons cultured from the neonatal rat superior cervical ganglion (SGG) express both muscarinic and nicotinic cholinergic receptors. We have been examining the regulation of cholinergic receptors by signals in the neuronal environment, which have been shown to affect neurotransmitter expression. For example, a membrane-associated factor (MANS) (Kong and Kessler, 1987) which stimulates cholinergic acetylcholine activity and decreases muscarinic receptor number in SGG neurons caused a large reduction in the number of muscarinic receptors and a corresponding decrease in muscarinic M2 mRNA levels. By contrast, no change was observed in levels of nicotinic receptors or in levels of nicotinic receptor M3 or M2 subunit mRNAs. Similarly, a soluble factor produced by rat fibroblasts (RFM) which also stimulated ChAT activity and decreased muscarinic receptor number in SGG neurons had no effect on levels of nicotinic receptors or their mRNAs. These data suggest that nicotinic receptor function may not be regulated by changes in receptor number, and that regulatory mechanisms for muscarinic and nicotinic receptors in the SGG are fundamentally different. We are currently examining the regulation of M2-adrenergic receptors and receptor mRNA levels by similar microenvironmental signals.


Muscarinic ACh receptors (mACHRs) in cerebral cortex slices are downregulated by increasing neural activity with veratridine or by activating the receptor with carbachol (Michael et al., Mol. Brain Res., 7, 61-68). The effect can be blocked by the K+ channel blockers TEA and apamin. To investigate the role of intracellular messengers (IM) in receptor downregulation, slices were incubated for 4h at 37°C with a wide variety of drugs acting on IM before being incubated for 2h at 4°C with [3H]NMS to label surface mAChRs. Forskolin and cholera toxin increased mAChR number. The effect can be blocked by the K*-channel blockers TEA and 83. To investigate the role of intracellular messengers (IM) in receptor downregulation, slices were incubated for 4h at 37°C with [3H]-NMS to label surface mAChRs. Forskolin and cholera toxin increased mAChR number. The effect can be blocked by the K*-channel blockers TEA and 83.


Muscarinic ACh receptors (MACHRs) in cerebral cortex slices are downregulated by increasing neural activity with veratridine or by activating the receptor with carbachol (Michael et al., Mol. Brain Res., 7, 61-68). The effect can be blocked by the K+ channel blockers TEA and apamin. To investigate the role of intracellular messengers (IM) in receptor downregulation, slices were incubated for 4h at 37°C with a wide variety of drugs acting on IM before being incubated for 2h at 4°C with [3H]NMS to label surface mAChRs. Forskolin and cholera toxin increased mAChR number. The effect can be blocked by the K*-channel blockers TEA and 83. To investigate the role of intracellular messengers (IM) in receptor downregulation, slices were incubated for 4h at 37°C with [3H]-NMS to label surface mAChRs. Forskolin and cholera toxin increased mAChR number. The effect can be blocked by the K*-channel blockers TEA and 83.

ACCELERATION OF CENTRAL MUSCARINIC RECEPTOR TOLERANCE BY INDOMETHACIN. A.C. Hays*, M.H. Sullivan*, W.C. Hallows* and J.J. Buccafusco (SPON: G.O. Carrier). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Our previous studies have demonstrated a significant tolerance to the hypertensive response to carbachol (CAB) and decreasing muscarinic receptors number. Opposite effects were obtained with the amount of CAB binding sites at the same concentrations of carbachol. On the other hand when cultures were incubated in presence of increasing concentrations of TPA, the value of scopolamine binding sites increased significantly. We don't understand why the muscarinic binding sites increased in presence of TPA however, this finding suggest a functional coupling in both directions between muscarinic receptors and protein kinase C.


Past studies have indicated that muscarinic acetylcholine receptors (mACHRs) are regulated by Estradiol (E2) and Pregesterone (P) in specific areas of the rat brain. In this study, the effects of E2 and P on mACHRs in female rat cortical slices were investigated to examine the relationship between the onset of puberty and the peak of mACHR binding on day 30-32 postnatal for the rat cerebral cortex.

Groups of 6-8 female littermates of similar weight (50 gms) were ovariectomized at 22 days of age. All 28 days of age the animals were sacrificed. Using a modification of the method of van Huizen et. al. (1989) Mol. Brain Res., 5, 59-69, 400mm thick cortical slices were preincubated for 90 minutes at 37°C with: E2 (10-10M), P (10-9M), E2+P (10-8M), or control (C). The slices were then incubated for 2 hours at 4°C with [3H]-N-methyl-scorpomine in increasing concentrations (0.15nM-40nM) to obtain saturation binding curves. The present results suggest that ovariectomy increases mACHR binding at day 30-32 postnatal for the rat cerebral cortex.

The addition of the gonadal hormone E2 in vitro, decreases MACHR Bmax by 20% from ovariectomy alone. P addition alone shows a similar decrease. The addition of both E2 and P increases the Bmax by a factor of 1.2. The Kd values for E2 are decreased when compared to ovariectomy alone (27%), P alone decreased the Kd only slightly less than E2 while P and E2 decrease more than E2.

RECIPROCAL INTERACTION BETWEEN M-RECEPTOR AND PROTEIN KINASE C IN CULTURED NEURONS FROM HIPPOCAMBUS. V. Aleman, S. Osorio* and J.L. Camacho*. Dept. of Physiology. CINVESTAV-IPN, Mexico City, Mexico.

Ten-day-old rats from both sexes were decapitated hippocampi dissected and their neurons dissociated and cultured during 10 days. Neurons were then treated during 24 hr adding either different amounts of carbamol (0.2, 5, 10, 20, 50, 100, 750, and 1,500 mU) or different concentrations of 12-0-tetradecanoylphorbol-13-acetate (TPA) 0.1, 1, 10, 30, 40, 100, and 200 nM. After this time, cells were harvested and P2 fraction obtained. Determination of muscarinic receptor was carried out using 40 nM of normin sample and 10 nM [3-methyl-3H] scopolamine, nonspecific binding was determined in presence of 10 nM atropine. For the determination of [3H]Phorbol-12-13 dibutyrate ([3H] PDBu) binding sites, a protein sample of 300 ng in presence of 60 nM concentration of [3H] PDBu was used. In these studies we found a relationship between increasing concentrations of carbachol and decreasing muscarinic receptors number. Opposite effects were obtained with the amount of [3H] PDBu binding sites at the same concentrations of carbachol. On the other hand when cultures were incubated in presence of increasing concentrations of TPA, the value of scopolamine binding sites increased significantly. We don't understand why the muscarinic binding sites increased in presence of TPA however, this finding suggest a functional coupling in both directions between muscarinic receptors and protein kinase C.

Chronic nicotine treatment results in tolerance development and receptor down-regulation. The combined use of two other nicotinic analogs, anabasine and lobeline, was tested for their effects on tolerance development and receptor regulation. Equimolar concentrations of anabasine, lobeline, and nicotine or saline were continuously infused into C57Bl/6 mice for nine days. Tolerance tests (respiration, y-maze crosses and rears, startling response, heart rate and body temperature) were then conducted following a challenge dose of the infused drugs. Tolerance was observed in the nicotine treated mice but not in the other treatment groups. Cross-tolerance was examined and results indicate a lack of cross-tolerance between any of the nicotinic analogs. Microdissection of the brains and receptor assays indicated an upregulation of nChR in all drug treatment groups but no changes in muscarinic receptors were seen. This study further suggests that the nChR following chronic agonist treatment is a property of the receptor itself and is not just specific to nicotine.

Supported by DA-001314 and DA-001161.

MELOTONIN INHIBITS [35S]-TBPS BINDING IN RAT BRAIN. L. P. Niles. Dept. Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 35.

In order to further clarify melatonin's interaction with the GABA-benzodiazepine (BZ) receptor complex, its effects on the binding characteristics of the GABA agonist, [3H]-BZ, a radiolabeled agonist, and the antagonist [3H]-TBPS (t-butylbicyclophosphorothionate), which binds to recognition sites on or associated with GABA-gated chloride channels were examined. Binding assays were carried out with fresh or frozen rat brain membranes. Non-specific binding was measured in the presence of 100uM GDP. In washed membranes, binding was maximally inhibited by 78% and melatonin had an IC50 of ~250uM. In washed membranes, binding was maximally inhibited by 78% and melatonin had an IC50 of ~200uM. A comparison of melatonin with various trypamine and catecholamines indicated that only melatonin consistently inhibited [3H]-TBPS binding. Saturation binding experiments conducted with or without melatonin (10 or 100uM) indicated that its effect is due to a decrease in the density of [3H]-TBPS binding sites with a concomitant increase in binding affinity. These findings indicate that melatonin allosterically inhibits TBPS binding as recently reported for GABA-positive BZ's, like diazepam. (Supported by the CMHF and MRC Canada.)


Solid and suspension grafts of fetal CNS tissue rapidly reform an intact BBB. Unlike normal agonist treatment, nicotine treatment upregulates the nicotinic cholinergic receptors (nChR). This upregulation could be specific to the agonist nicotine itself. A property of the survival of the grafted tissue and the neural environment surrounding the graft. However, cell suspension of SCG formed a BBB by seven days. These findings indicate that the nChR following chronic agonist treatment is a property of the receptor itself and is not just specific to nicotine.

Multidrug-resistance refers to the ability of a cell to become less permeable and to result in a failure of chemical interaction in the P-glycoprotein (P-GP) binding site. The development of drug resistance in cancer cells has been associated with the development of multidrug-resistant cells. The mechanism of multidrug resistance has been attributed to the development of drug resistance in cancer cells to multiple pharmacologic agents. Mianserin is an antidepressant that has been shown to be effective in the treatment of depression and anxiety. The mechanism of action of Mianserin is not fully understood, but it is thought to act as a selective adrenergic agonist. The results of this study suggest that Mianserin may provide an additional line of defense at the level of the blood-brain barrier. Supported by NS32324 to L 林.


The rate of entry of albumin into the endoneurial space and its fate within that compartment were investigated by measuring the permeability coefficient-surface area product (PS) of the blood-nerve interface (BNI) to 125-albumin, residual endoneurial plasma volume (Vp), and the BNI index to albumin in sciatic nerves of 1, 2, 3, 4, 6, 8, and 13 week old rats. The results are given in Table 1. The results are as follows:

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>PS (mL/g/10^6)</th>
<th>Vp (%)</th>
<th>BNI Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.80±0.3</td>
<td>3.90±0.5</td>
<td>3.410±0.08</td>
</tr>
<tr>
<td>2</td>
<td>7.60±0.6</td>
<td>3.20±0.04</td>
<td>3.29±0.04</td>
</tr>
<tr>
<td>3</td>
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<td>3.420±0.2</td>
<td>3.26±0.02</td>
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</tr>
<tr>
<td>8</td>
<td>10.2±0.1</td>
<td>3.12±0.2</td>
<td>3.26±0.02</td>
</tr>
<tr>
<td>12</td>
<td>6.80±0.1</td>
<td>3.00±0.2</td>
<td>3.26±0.02</td>
</tr>
</tbody>
</table>

At two weeks, compared to 13 weeks, there is a transient increase in albumin in the endoneurium, and this decreases towards adult values as both components of BNI become less permeable. (NHL13405, NSW92323, and Borchard Fund).


Tricyclic antidepressants potentiate the P-gp (P-glycoprotein) of the blood-brain barrier (BBB) to facilitate the entry of macromolecules into the central nervous system. Minerserin is an antidepressant that increases the efficacy of alpha and alpha agonist without retarding blockade. A 2x2x2 factorial design was used to study the effects of minerserin on cerebral blood flow (CBF) function. Sprague-Dawley rats were divided into 6 groups, with varying conditions of CO2 (5%, 7%, 10%, 15%, 20%, and 30%) and varying conditions of NaHCO3 (0, 0.1, 0.5, 1, and 2 M). In all groups, minerserin dose was 0.001, 0.01, 0.1, and 1 M. Minerserin did not affect arterial blood gases or CO2 tension at any dose, but significantly decreased the normal tight coupling of CBF to CO2. The results are as follows:

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Tricyclic antidepressants (TCAs) increase blood brain barrier (BBB) permeability to water and affect volume and composition of brain extracellular fluid compartment via adrenergic mechanisms. This study evaluated changes in brain ultrastructure and water content coincident with PS changes. Amitriptyline (AMI) 15 and 30 mg/kg dw was given intraperitoneally to rats under acute (15 min. before sacrifice, n=6) and chronic (daily x 10 days, n=6) conditions. Half of the chronically treated animals received an acute injection. The anesthetized and heparinized animals were sacrificed after intra-cardiac perfusion fixation. Tissue was processed for light and electron microscopy. The same design tested AMI effect on brain specific gravity, measured by the gradient column method. There were controls for both. Electron microscopy was used to determine if BBB changes characteristic of increased permeability to macromolecules in cells with increased pinocytic activity and of fibrillary astrocytes particularly their perivascular foot process were seen. There was no disruption of the BBB integrity and no neuronal changes. There was no regional changes in brain specific gravity. PS changes are associated with physiologically significant but reversible changes in fluid dynamics and could underlie ability of norepinephrine to modulate the response of neurons in remote areas to afferent input.
**480.9**  
RAPID SEALING OF BLOOD-BRAIN BARRIER LEAKS AFTER ETHANOL PLUS BARBIRRITATE-INDUCED DAMAGE. P.A. Stewart, J.A. Rolak*, C.R. Parrelli* and W.M. Naylor*, Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, Canada. M5S IAB  
Ethanol in high doses induces multifocal leaks in the blood-brain barrier (BBB) leading to a population of brain endothelial cells. Administration of thiopept, a commonly used anesthetic in humans, and energy drink with ethanol to cause similar barrier damage at much lower doses of ethanol. The time course of barrier breakdown and recovery was investigated. Using horseradish peroxidase (HRP) - a vascular tracer that does not cross the intact BBB. Tissue section were stained for HRP, measured in cerebral cortex, hippocampus and cerebellum. HRP was significantly elevated in all areas 3 minutes after drug administration, and remained high for at least one hour. By two hours total HRP had decreased to control levels, where it remained at least to six hours. This rapid breakdown of the barrier is consistent with gross structural damage to endothelial cells seen previously. The rapid rate of sealing in barrier leaks suggest that fibrin clot formation initiated by endothelial lysis may be responsible. Supported by MRC Canada.

**480.10**  
ALTERATION OF BLOOD-BRAIN BARRIER PERMEABILITY IN A BACTERIAL CEREBRITIS. Warren D. Lo and David McNeely*, Dept. of Pediatrics, Ohio State Univ., Columbus, OH 43210.  
Inoculation of Staph. aureus (S. aureus) into the rat brain induces an influx of neuropsilin into the brain within 4-6 hours. We have reported that in this model abnormal cerebrovascular permeability was assessed by leakage of Evans Blue, does not develop until day 3 in the S.aureus inoculated brain (Soc. Neurosci. 13:949, 1987).  
We measured gray matter specific gravity as an indication of tissue water content. On day 4 the specific gravity of S. aureus-inoculated cortex was 1.0455+0.0026 (n=11) vs. 1.0455+0.0007 (n=11) in control cortex (p<0.05), thus water accumulated in the inoculated cortex. We used 125I-2,4,6-Tricose as a marker to measure the brain permeability-surface area product (PS) in anesthetized rats. The PS of the inoculated region (in ml/min/mg x 10^-3) on day 0 was 2.92+/-0.56 (n=7) (experimental animals) vs. 2.64+/-1.34 (n=6) (controls) (N.S.). Thus, when Evans Blue leakage demonstrates increased blood-brain barrier permeability, there is no net movement of the tracer that does not cross the intact BBB. We speculate that in a cerebritis, the nature of an intravascular tracer (i.e. polarity or charge) determines its movement into and retention by the brain.

**480.11**  
BLOOD-BRAIN BARRIER (BBB) TRANSPORT OF CATIONIZED IMMUNOGLOBULIN G: ENHANCED DELIVERY COMPARED TO NATIVE PROTEIN. D. Triggle*, J. Buciak*, L. Yang*, and W.W. Pardridge* (SPO: E. Conford), Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.  
Immunoglobulin G (IgG) molecules are potential neuropharmaceuticals that may be used for therapeutic or diagnostic purposes. The possibility of enhanced IgG delivery through the BBB by cationization of the proteins was explored in the present studies. Native IgG molecules were cationized by the covalent coupling of hexamethylene diamine, and the isoelectric point was raised to >10.7 based on isoelectric focusing studies. Native or cationized IgG molecules greatly facilitates the transport of these plasma proteins through the BBB in vivo, or at paravascular cells such as smooth muscle or pericytes. The microvascular DR-antigen was localized by avidin -biotin-immunoperoxidase studies using a mouse monoclonal antibody to the human DR-antigen or a mouse myeloma IgG2a control, and microvessels isolated from either fresh or frozen autopsied human brain. The DR-antigen was readily detectable in precapillary arterioles in smooth muscle cells in all subjects, but was reduced in intensity and confined to a crescent-shaped capillary in patients with chronic scarring. The capillary plasma mem-brane fraction was isolated and the DR-antigen antibody precipitated a labeled 35K protein, which is identical to the molecular weight of the β-subunit of the human DR-antigen. Conclusions: These experiments show that the DR-antigen is readily detectable in human microvasculature of normal brain and is found in the smooth muscle cells of precapillary arterioles and in capillary pericytes with minimal, if any, staining of capillary endothelial. These results are consistent with the hypothesis that antigen presentation in the CNS occurs primarily at a site immediately distal to the blood-brain endothelial interface.

**480.12**  
HUMAN BLOOD-BRAIN BARRIER DR-ANTIGEN. J. Yang*, W.M. Pardridge*, J. Buciak*, and W.V. Tourtellotte* (SPO: D. Markham), Departments of Medicine and Neurology, UCLA School of Medicine, Los Angeles, CA 90024-6028.  
Antigen presentation within the human central nervous system (CNS) by the class II histocompatibility or DR-antigen may take place at either the brain-capsillary endothelial interface, which makes up the blood-brain barrier (BBB) in vivo, or at paravascular cells such as smooth muscle or pericytes. The microvascular DR-antigen was localized by avidin -biotin-immunoperoxidase studies using a mouse monoclonal antibody to the human DR-antigen or a mouse myeloma IgG2a control, and microvessels isolated from either fresh or frozen autopsied human brain. The DR-antigen was readily detectable in precapillary arterioles in smooth muscle cells in all subjects, but was reduced in intensity and confined to a crescent-shaped capillary in patients with chronic scarring. The capillary plasma membrane fraction was isolated and the DR-antigen antibody precipitated a labeled 35K protein, which is identical to the molecular weight of the β-subunit of the human DR-antigen. Conclusions: These experiments show that the DR-antigen is readily detectable in human microvasculature of normal brain and is found in the smooth muscle cells of precapillary arterioles and in capillary pericytes with minimal, if any, staining of capillary endothelial. These results are consistent with the hypothesis that antigen presentation in the CNS occurs primarily at a site immediately distal to the blood-brain endothelial interface.
481.3

Cutting the IO nerve in adults produces long-term somatosensory reorganization in rat trigeminal brainstem and nucleus complex (TMBC). Lesion-induced changes in monoamine systems may be a substrate for this reorganization, since these systems normally influence activity of TMBC neurons. Thus, we have used HPLC-ED to measure serotonin (5-HT) and noradrenaline (NE) levels in the IO region of subnuclear caudalis (SpVc) and interpolaris (SpVi) 76-79 days after unilateral adult IO nerve cut. Following decapsulation, brains were quickly frozen and 30 μm transverse cryostat sections were prepared. Samples of 1.2 x 2.2 μm were respectively sliced from the lateral border of SpVc & SpVi sections, in the IO region. Samples were taken from nerve cut & intact sides of each section in 9 lesioned rats unilaterally in 10 normals. Location of samples was histologically verified. In SpVc, no differences were observed in 5-HT or NE levels among the lesioned, intact or normal sides. However, in SpVi, 5-HT & NE levels were increased by 46% & 60%, respectively, compared with the matched intact side. The data for SpVi are consistent with our previous finding that the adult IO nerve cut does not alter the density of 5-HT immunoreactive varicosities in the superficial laminae. Analysis of monoaminergic fiber distribution within SpVc is in progress. Support: NIAAA-5M01-OM.

481.4
PLASTICITY IN THE RAT TRIGEMINAL SOMATOSENSORY PATHWAY: RETURN OF CORTICALLY-DEPENDENT BEHAVIOR FOLLOWING LESION OF MAIN V NUCLEUS. M.A. Freedom, F.B. Siegel, L.W. Evarts, and F.F. Ebner. Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.

The functional sensory cortex of the rat is normally organized in such a way that nociceptive input from the trigeminal subnucleus interpolaris (SpVpi) has little sensory processing role. However, if the spinal trigeminal complex of the cat is lesioned, the function of SpVpi as sensory relay is expanded. This study confirms that lesioning of main trigeminal nucleus (MainV) produces expanded receptive fields of ventroposteromedial (VPM) neurons. With this whisker configuration mice were allowed to explore, for 45 min, an object-filled cage, and/or PO. Following MainV lesions, 29% of BF neurons (N = 38) would then perform the gap-jump task during the initial 24 hr period. Thalamic recordings during this same period failed to reveal any neurons sensitive to vibrissae movements. Retesting each of the seven experimental animals after 24 hr demonstrated a marked increase in the success rate comparable to those of prelesion cases at gap distances shown to be dependent on integrity of the vibrissa somatosensory pathway. Subsequent destruction of SpVp after 10-20 d testing periods resulted in a permanent inability to use vibrissa-transduced information similar to the initial 24 hr period. In four control animals comparable injections of saline produced no behavioral or physiological deficits. Our results agree with those of Rhoades et al. that lesions of MainV produce expanded receptive fields of ventroposteromedial (VPM) neurons. Our results show that an even more dramatic expansion occurs in the SI cortical region ('barrel field'). Despite this apparent loss of acuity the animal can use the sensory information mediated through the SpVpi to make cortically-dependent decisions (Supported by NIH grant NS-10301 and the Mathers Foundation).

481.5
CHANGES IN RECEPTIVE FIELD PROPERTIES FOLLOWING RAT BARRELFIELD NEURONS FOLLOWING THALAMIC LESIONS. F.E. Ebner, M.A. Armstrong-James* and M.E. Barr. University, Providence, R.I. 02912.

In rats, two pathways conduct information from the mystacial vibrissae to the barrelfield (BF) cortex; one projects through the thalamic ventroposteromedial nucleus (VPM) to BF cortex; the other projects through the thalamo-cortical pathway. Subsequent destruction of SpVi after 10-30 days failed to reveal any neurons sensitive to vibrissae movements. Retesting each of the seven experimental animals after 24 hr demonstrated a marked increase in the success rate comparable to those of prelesion cases at gap distances shown to be dependent on integrity of the vibrissa somatosensory pathway. Subsequent destruction of SpVp after 10-20 d testing periods resulted in a permanent inability to use vibrissa-transduced information similar to the initial 24 hr period. In four control animals comparable injections of saline produced no behavioral or physiological deficits. Our results agree with those of Rhoades et al. that lesions of MainV produce expanded receptive fields of ventroposteromedial (VPM) neurons. Our results show that an even more dramatic expansion occurs in the SI cortical region ('barrel field'). Despite this apparent loss of acuity the animal can use the sensory information mediated through the SpVpi to make cortically-dependent decisions (Supported by NIH grant NS-10301 and the Mathers Foundation).

481.6

Neurons of the principal sensory relay in the mouse, the Lausanne whisker-stimulator (Meister et al., 1985), result in the expansion of GAD-immunoreactivity in the corresponding barrel cortex. The effect was present if slightly. After stimulation the effect wore off gradually until, five days after its arrest, GAD-immunoreactivity returned to normal. Our interpretation is that lesions affecting the normally dominant VPM input to BF cortex lead to enhancement of the normally undetectable PO influences, permitting cortex to respond to sensory stimuli in a novel way. (Supported by NIH grant NS-25907).

481.7

We investigated experience-dependent regulation of cortical activity in the whisker-to-barrel pathway of the adult mouse using the 2-deoxyglucose method. Under Nembutal anesthesia, metal pieces were glued on left whisker C3, with all other whiskers remaining intact. The mice were exposed to a magnetic field bursts while freely moving in the Lau­ sanne whisker-stimulator (Melzer et al. 1985). Activity-dependent regulation of neurotrans­ mission may be at the root of this phenomenon. Our interpretation is that lesions affecting the normally dominant VPM influence to BF cortex lead to enhancement of the normally undetectable PO influences, permitting cortex to respond to sensory stimuli in a novel way. (Supported by NIH grant NS-25907).

481.8

To explore treatments that maximize functional cortical alterations, this study combined a bilateral spared vibrissa preparation with unilateral associatively-paired (AP) training in 5 rats. Subtotal deafferentation involved bilateral sparing of C3 vibrissae (SC3) before postnatal day 3. AP training (classically pairing vibrissa stroking with sugar water) of left or right SC3 was continued for 5 min/day for 60 days. Using the quantitative 2DG metabolic technique, results reveal a significant (p<0.05) increase in SC3/AP cortical area of 34.9% (+14.1,SE). Does increased cortical area mean enhanced Sensory processing? Behavioral testing involving 5 days of 4 minute trials using a darkened, raised circular maze indicates that 6 of 8 C5 rats performed better in the predicted direction preferring to use the SC3/AP-trained vibrissa in dark exploration. The data suggest that inter­ vention resulting in significant functional cortical alteration also results in behavioral observations that can be quantified. Correlation of sensory training, changes in functional brain activity, and behavioral out­ come is therefore possible in the same experimental animal. NIT 22283-03.

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THURSDAY PM

1222 NEURAL PLASTICITY IN ADULT ANIMALS: SENSORY SYSTEMS

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CORTICAL REPRESENTATION OF THE BODY FOLLOWING COMPLETE DEAFFERENTATION OF THE FORELIMB IN ADULT CATS. R.J. Schneider1, Barth, & Schallert, 1988). That is, a second lesion placed in the homotopic cortex (hCF) is especially vulnerable to damage (Jones, et al. 1988). Consequently, we have studied by means of simultaneous recordings correlated unit responses in both forepaws of SI in rats and in the hand representations of area 3b in owl monkeys while inducing local cortical representational changes by intracortical microstimulation. The present experiment examined the alterations in CORR ( corticated representation) at separated locations in the forepaw zone of SI in adult cats 60 days postoperative at several positions within and neighboring the post-sigmoid gyrus. We found parallel time course for changes of CORR, RP-overlaps, correlation of PSTHs, and onset latencies. Changes were first seen after 15 to 30 min of ICMS. Steady state conditions were reached after 2 to 3 hours, and remained robust over subsequent studies of at least 2 weeks.

These studies suggest that temporal discommodation plays an important role in the formation of functionally coupled cortical neuron groups and implicates these functionally groups in representational plasticity. They provide further evidence for a long term potentiation induced by ICMS.

FREQUENCY DISCRIMINATION TRAINING ALTERS TOPOGRAPHICAL REPRESENTATIONS AND DISTRIBUTED TEMPORAL RESPONSE PROPERTIES OF NEURONS IN PRIMARY SOMATOSENSORY CORTEX OF THE ADULT CAT. J. S. Schneider1,2,3, S. E. Water4, T. Schallert1,2. We report the consequences of sectioning the ulnar nerve on the topographic organization of somatosensory cortex in cat. This study was undertaken to lay the groundwork for ongoing studies on the mechanisms underlying the unmasking phenomena after peripheral denervation.

Thirty-two cats were used. Ten maps of the primary somatosensory cortex were obtained in unoperated animals. In the remaining animals, the median, radial, and ulnar nerves serving the forelimb. This information will provide the groundwork for ongoing studies on the mechanisms underlying the unmasking phenomena after peripheral denervation.

To investigate mechanisms underlying the remodeling of topographic representations by use, we have studied by means of simultaneous recordings correlation of unit responses in both forepaws of SI in rats and in the hand representations of area 3b in owl monkeys while inducing local cortical representational changes by intracortical microstimulation. The present experiment examined the alterations in CORR (corticated representation) at separated locations in the forepaw zone of SI in adult cats 60 days postoperative at several positions within and neighboring the post-sigmoid gyrus. We found parallel time course for changes of CORR, RP-overlaps, correlation of PSTHs, and onset latencies. Changes were first seen after 15 to 30 min of ICMS. Steady state conditions were reached after 2 to 3 hours, and remained robust over subsequent studies of at least 2 weeks.

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481.15

ACTIVITY-DEPENDENT PLASTICITY IN THE MATURE RAT GENICULO-
STRATE SYSTEM DURING MONOCULAR RETINAL BLOCKADE.
G.A. Thrulow and R.M. Cooper. Behavioral Neuroscience Research
Group, Psychology Dept., University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

We examined the effects of monocular loss of retinal activity on 2-deoxyglucose (2-DG) uptake in the mature
hooded rat geniculostriate visual system. Rats were
subjected to either short-term (24 h) or long-term
(21 - 90 days post 2-DG uptake). Ultrastructural analyses revealed increases in
the number of synapses on 2-DG uptake. As compared to short-term TTX-rats, 2-DG uptake increased in
the ipsilateral TTX injections and exposed to a
visually stimulating environment during 2-DG uptake. We conclude that adult retinal activity was
eliminated. Complete loss of input resulted in
local changes occurring in the binocular geniculate.
During long-term monocular retinal blockade in the mature rat, an
activity-dependent shift in ocular dominance occurs in the
geniculostriate visual system, increasing the meta-
481.16

RAPID MORPHOLOGICAL CHANGES IN THE NEURONAL IMMUNOREACTIVE CELLS IN THE HAMSTER'S SUPERIOR COLICULUS.
R.W. Phoakides, C.A. Bennett-Clarke, N.L. Chiara and R.D. Monney. Dept. of
Anatomy, Medical College of Ohio, Toledo, OH 43669.

The stratum griseum superficiale (SGS) of the hamster's superior colliculus
contains a substantial number of cells that are recognized by polyclonal antisera directed against serotonin (Inc Star and Accurate). These neurons can be seen in animals that are pretreated with pargyline only, but are much more numerous after pretreatment with both colchicine and pargyline. Pretreatment with reserpine causes the complete depletion of all serotonin-immunoreactive (SI) cells in the hamster's SC. In this experiment, we asked whether the presence of SI neurons in the hamster's SC could be modulated by the optical input to this nucleus. Adult hamsters were anesthetized with sodium pentobarbital and either one or both eyes were enucleated. One day to 2 months later, hamsters were pretreated with pargyline and colchicine, killed, and tissue was processed for SI. In hamsters that sustained removal of one eye, there was a virtually
481.17

GAP-43 EXPRESSION IN REGENERATING ADULT OPTIC FIBERS IN VITRO. J. Miotke*, R.L. Meyer and L.I. Benowitz
(SPON: G. LeBlanc). Developmental Biology Center, Univ. Calif.,
Irvine, CA 92717 and Dept. Psychiatry, Harvard Med. School,
Belmont, MA 02178.

We previously reported that when the optic nerve is crushed in an
adult mouse and 1wk later the retina explanted onto laminin, neurons originating from ganglion cells extend onto the substrate
as early as 24h. If the optic nerve is not crushed, neurites begin to
reach the substrate by about 4d. In this study, the expression and distribution of GAP-43 was examined using immunohistochemistry. In explants with prior nerve crush, all neurites were strongly positive as early as 24h and remained positive for up to 1wk. When the explants, numerous processes and some cell bodies were also positive. In explants without crush, no positive processes or cell bodies could be detected at 24h: they were seen at 4-6d. Strongly positive
481.18


Magnocellular neurons in the rat SON alter their ultrastructural morphology in response to dehydration of the animal in vivo. To determine if similar changes occurred in vitro, horizontal hypothalamic slices that contained the SON were
incubated in normal (290 mOsm/kg, n=9) or high
(340 mOsm/kg, n=8) osmolality medium. Electrical activity was
monitored extracellularly from samples of neurons and mean firing rates were obtained. A significant increase in mean firing rate was found in the slices incubated in high osmolality medium. Ultrastructural evaluation revealed that incubation in such medium for 4.5 hours resulted in the formation of new
terminal-like branches that contain numerous synaptic
dendrites. These results demonstrate the suitability of this slice preparation for further studies of the
mechanisms through which the SON is reorganized. The rapidity of this response suggests that pre-existing terminals either within or around the SON are forming these new synapses. These
synapses are thought to play an important role in the overall responsiveness of the SON to osmotic challenge. Supported by NIH NS 09140.
SENSITIZATION OF DOPAMINE RELEASE TO INJECTIONS OF COCAINE AS MONITORED BY IN VIVO MICRODIALYSIS.

R.W. Keller, Jr., M.M. Malenconneve*, J.N. Carlson, S.G. Glick. Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208

We have examined changes in the levels of dopamine (DA) and its metabolites in the extracellular fluid of the striata of freely-moving, male, Long-Evans rats in response to i.P. injections of 20 mg/kg cocaine. Dialysis probes (Carnegie Med.) were lowered into previously implanted guide cannulas and perfused at 1 ul/min. 20-min samples were assayed by HPLC-EC for DA, DOPAC, HVA and 5-HIAA. Baseline samples were collected before 10, or 22 hrs after probe insertion. Saline or cocaine was injected 2 hrs later and a stable baseline cocaine profile was obtained. All rats were reexamined one week later; in each case baseline collection began 2 hrs after probe insertion and cocaine was injected 2 hrs later.

Basal extracellular fluid levels, estimated from pre-implantation calibration of the probes, were 2.0M DA, 6.5M DOPAC, 5.3M HVA and 2.8M 5-HIAA. A 6-fold increase in DA was observed after cocaine while DOPAC, HVA and 5-HIAA showed modest decreases. We also studied infant rats (10-14 days) and found lower basal levels of all compounds and a more prolonged release of DA in response to cocaine. In adult rats, one week after cocaine, basal DA doubled, while it was unchanged in saline-injected controls. Therefore, the response to the second administration of cocaine was also enhanced. Thus a single exposure to cocaine appears sufficient to produce a sensitization of DA release measurable in vivo. [Support DA-03817]


The accumulation of 3-methoxytyramine (3MT), a reflection of dopamine release, was measured in the frontal cortex (Fx) and striatum (ST) following acute and chronic clozapine (CLZ) and haloperidol (HAL) treatment. Rats received HAL (0.4 mg/kg), CLZ (10 mg/kg) or vehicle (VEH) i.p. daily for 28 days, and were killed by microwave 10 min after pargyline (165 mg/kg) and 1 hr (CH-1hr) or 24 hr (CH-24hr) after final drug injection. 3MT was measured by mass fragmentography.

In the Fx, a single dose of CLZ or HAL following chronic VEH elevated 3MT (p<.05). In the CH-1hr group, both drugs elevated 3MT, although not as the single dose (p<.05), suggesting partial tolerance. In the CH-24hr group, 3MT returned to baseline. In the ST, after chronic VEH, HAL increased 3MT; however, for CH-24hr dosing, 3MT was well below baseline (p<.05). It appears that there is partial tolerance for both CLZ and HAL in the Fx. In the ST, there is no tolerance to HAL. In contrast, CLZ fails to increase ST 3MT above baseline, but decreases the 24 hr baseline. The clinical significance of these findings will be discussed.

EFFECTS OF ACUTE AND CHRONIC HALOPERIDOL AND Clozapine ON Dopamine RELEASE IN THE FRONTAL CORTEX AND STRIATUM.


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A RAPID MICROSAMPLING TECHNIQUE DESIGNED FOR THE INTERFACE DOPAMINE METABOLISM IN THE MEDIAL PREFRONTAL CORTEX (M PFC) OF THE RAT. The technique has been shown to measure extracellular levels of several compounds in freely moving rats. Dialysis probes were consecutively perfused with Dulbecco's phosphate buffer containing 1.2 mM CaCl2, pH 6.0, at a rate of 2.5 µl/min. Dialysate samples were collected every 20 min, split into two aliquots and analyzed by HPLC EC for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) or aspartate, glutamate, taurine and GABA. After extraction, the samples were immediately analyzed with the rapid sampling technique.

EFFECTS ON EXTRACELLULAR CAUDATE DOPAMINE (DA) AS MEASURED AT A RAPID RATE OF 2.5 µl/min. Administration of FG-7142 caused a peak at 60% of baseline concentration within 20 min of injection, followed by a return to baseline values within 60 min post-injection. This result suggests that benzodiazepines can increase the electrophysiological activity of DA neurons, and that the effect is not necessarily be associated with an increase in extracellular DA at the level of the terminals.

INCREASED DA RELEASE IN PREFRONTAL CORTEX OF AWAKE FREELY MOVING RATS BY THE ANXIOLYTIC BETA-CARBOXYLIC ACID. J.M. Finlay.

EFFECTS OF THE BENZODIAZEPINE MIDAZOLAM ON EXTRACELLULAR DOPAMINE CONCENTRATIONS IN THE NUCLEUS ACCUMBENS AND STRIATUM. J.M. Finlay.

INVESTIGATION OF ELECTROCHEMICALLY STIMULATED RELEASE FROM THE LOCUS COERULEUS-NOREPINEPHRINE SYSTEM IN THALAMUS USING IN VIVO VOLTMETRY. M. Nasir, A. Chamseddine, R. Capella, R.N. Adams. Dep. of Chemistry, University of Kansas, Lawrence, KS 66045.

Nafion-coated carbon fiber electrodes (CFE) were used to study the locus coeruleus (LC)-norepinephrine (NE) system. We have electrically stimulated (10 sec., 50-100 µA) both LC and its ascending dorsal bundle, and have recorded a "fast" signal (30-60 sec., duration) followed by a "slow" signal (2-5 min., duration) in the anterior ventral nucleus of thalamus in anesthetized rats. We believe the "fast" signal to be composed of extracellular catecholamine(s), and the "slow" signal, catecholamine metabolite(s): (1) the location of the stimulating electrode and CFE must be in known NE tracks and terminal fields to observe the release; (2) the applied potential was sufficient to oxidize catecholamines, not indoleamines; (3) inhibition of tyrosine hydroxylase reduced both signals by 50-70%; (4) inhibition of monoamine oxidase eliminated the "slow" signal and increased the "fast" signal 3-4 fold; (5) inhibition of NE uptake by desipramine increased and broadened the fast signal by 2-3 fold. This is the first report of real time, in vivo monitoring of release and metabolism of neurotransmitters from the LC-NE system.

The mechanisms underlying presynaptic receptor modulation of dopamine (DA) release have not been clearly defined. Modulation of endogenous DA release (assayed by HPLC-EC) from rat striatal slices was studied. Slices were superfused with Krebs' buffer containing 10 μM nomifensine at a rate of 1 ml/min. One ml samples were collected before and after the two periods of stimulation. Drugs were added 15 min before the second period of stimulation. Adenosine (50 μM) inhibited evoked DA release by 30-40%. This effect was apparently mediated via an A1 adenosine receptor because the A1 specific agonist N6-cyclohexyladenosine (100 nM) produced a similar inhibition while the A2 specific agonist CGS 21680 (100 nM) had no effect. The inhibition of DA release caused by adenosine was abolished by co-perfusion with the adenosine receptor antagonist 8-phenyltheophylline (10 μM). p-Bromophenacyl bromide (BPA) irreversibly inhibits phospholipase A2 and thus prevents release of arachidonic acid (AA) from membranes. Co-perfusion with BPA attenuated, in a dose dependent manner, the inhibition of evoked DA release produced by adenosine. 10 μM BPA produced a maximal effect. BPA (10 μM) by itself tripped the basal release of DA, suggesting a role for AA metabolites in the control of basal release of DA, as well as in the modulation of stimulated release. The AA cascade may be a general mechanism involved in the presynaptic inhibition of striatal DA release, as BPA could block the inhibition of DA release caused by the D-2 DA receptor agonist N-0437 (3 μM). Supported by USPHS NS26851 and AA07464.

482.15 PROLACTIN MODULATES Dopamine RELEASE FROM THE CORPUS STRIATUM THROUGH A SPINERONE BINDING SITE IN THE ABSENCE OF extracellular calcium. R.J. Lapin, D.B. Uijesen, and V.D. Ramirez (Dept. of Physiology and Biophysics, Denver, CO 80262).

In vitro basal and amphetamine (AMPH) stimulated dopamine (DA) release from corpus striatum homogenates of 4-8 month old male rats was measured in the presence of 10-4, 10-5, and 10-6 M ovine prolactin (PRL). Additional conditions included 10-5 M Ca++, in calcium-free medium, 10-6 M spiperone, and 10-5 M PRL in the presence of 10-6 M spiperone. PRL increased basal DA release in a dose-dependent fashion with 10-5 M PRL being the most effective (232±5 vs 624±239 pg/mg; control vs PRL, p<0.05). AMPH-stimulated DA release in a dose-dependent manner with 10-5 M PRL being the most effective 194±55 vs 878±273 pg/mg; control vs PRL, p<0.05). Also, spiperone blocked the PRL-induced stimulation of AMPH-stimulated DA release (2048±7247 pg/mg). It was noted that the effect of PRL on basal DA release is on D2 DA autoreceptors and that PRL interferes with AMPH-stimulated DA release via a spiperone site.


Thyrotropin-releasing hormone (TRH) receptors are found in high densities in limbic structures. Some TRH receptors may be located on dopaminergic (DA) nerve terminals. Since 6-OHDA causes a decrease in the number of TRH receptors, furthermore, TRH enhances locomotor activity when injected into the nucleus accumbens (NAC). Finally TRH and the TRH analogue CG 3509 cause an increase in DA function. In the present study, the effects of CG 3509 on basal levels of DA as well as on K+-evoked DA release were examined using in vivo electrochemistry. Chloral hydrate anesthetized rats were placed in a stereotaxic frame. A guide cannula was implanted into the lateral ventricle and a Narbon-coated, graphite-pyrex ceramic voltmeter electrode was lowered into the NAC. Electrochemical measures were obtained by applying to the voltmeter electrode at a rate of 15 Hz, a 0.5 V pulse in relation to a Ag/AgCl reference electrode. The currents resulting from the oxidation of electroactive species was digitized and graphically displayed on a video monitor. Local K+ stimulation was accomplished by pressure ejection of a 128 mM KCl solution from the tip of a pulled glass capillary cemented 200-250 μm from the tip of the voltmeter electrode. Intraventricular infusions of CG 3509 (3-30 μg) had only negligible effects on the basal electrochemical signal even at the highest dose. However, both the magnitude and the duration of K+-evoked electrochemical signals were potentiated by CG 3509. The potentiating effect of CG 3509 was observed 45 min after injection and appeared to be maximal 90-120 min after injection. We are presently examining the effects of CG 3509 on DA release in other terminal fields. In conclusion the present data are consistent with the idea that TRH enhances dopaminergic function. Supported by Medical Research Council of Canada grant MA-10739.


Bombesin (BN) and neuropeptide Y (NPY) are neurochemically related agents which, both in vivo and in vitro, affect motor activity in rats. The caudate-putamen (CPU) and the nucleus accumbens (ACB) represent regions of the brain that are particularly rich in dopaminergic (DA) input, BN binding sites and NPY levels. The effect of BN and NPY on DA and its metabolites (DOPAC, 3-MT and HVA) were compared to those of d-amphetamine (d-AMPH). Sprague-Dawley rats were implanted with microdialysis assemblies with guillotine shafts aimed at the Acb and the CPU (Brain Microdev Inc., Box 410, Station A, Ottawa, KIN 8V4). After steady baseline, BN (40 μg), NPY (30 μg) or d-AMPH (40 μg) was pulsed through the probe (30 μl/20 min) and samples collected for 2 hr. At both sites, BN and NPY increased levels of DA. This was followed by smaller but more prolonged increases of DOPAC and HVA. d-AMPH markedly increased DA but decreased DOPAC and HVA levels. 3-MT levels that were barely detectable at baseline were not increased after BN or NPY. Thus both BN and NPY can increase the extracellular levels of DA most likely by releasing DA and/or release of endogenous dopamine (DA) by LC-EC synapses enabled us to dissociate DA synthesis from DA release. In vivo studies of basal NE levels, we also observed alterations in its metabolism, (independently of extracellular Ca**). (±)amphetamine, reserpine and 6-OHDA decreased NE levels, whereas high K+ evoked NE release was reduced to control levels. Our data indicate that studies using the rabbit retina in vivo, may lead to a better understanding of the regulation of dopaminergic activity at various synaptic sites.
482.19


We have examined the effect of local application of glutamate (GLU) on in vivo release of endogenous dopamine (DA) in the rat striatum (CP). In one group of experiments, dialysis probes were lowered bilaterally into the CP and recovered DA was assessed with HPLC-EC. In another group of experiments, ion-selective and Nafion coated carbon fiber microelectrodes were used to simultaneously monitor extracellular K+ and DA levels. In the dialysis experiments, local infusion of 0.5 mM GLU led to a significant decrease in basal DA release. Perfusion of 1µM, 50µM, 100µM, or 500µM GLU had no significant effect. Furthermore, local application of 500 µM GLU did not augment the K+-stimulated release of DA. In the electrochemical studies, microperfusion injections of approximately 100 nl of 0.1 mM and 1 mM GLU led to an increase in extracellular K+ but not DA. Microinjection of 10 mM GLU, accompanied by a large increase in extracellular K+ (20-50 mM), led to an increase in DA levels. It is likely that GLU mediated increase in DA was an indirect effect caused by a massive depolarization or initiation of spreading depression since it was accompanied by a massive outflow of K+. The dialysis and electrochemical data do not support the notion that GLU has an excitatory effect on the release of DA from nigrostriatal terminals. This work was supported in part by the following grants: MH14092; DA 05119; MH14276; and, MH25842.

482.20


In addition to its direct effect on anterior pituitary hormone secretion, previous evidence has suggested that thyrotropin-releasing hormone (TRH) may also alter central nervous neurotransmission, particularly that mediated by the monoaminergic systems. We have examined the effects of systemically administered TRH on striatal dopamine release in the anesthetized rat in vivo (using brain microdialysis) and in vitro (using striatal homogenates). Animals (microdialysis n = 7; homogenates n = 3) received TRH (10µg or 50µg iv) and/or tyrosine (as methyl-ester, 20mg/kg iv) or saline placebo. After probe implantation and when dopamine release had stabilized, the drugs were administered through the jugular catheter, and microdialysis samples (15min; 1.5µl/min) were collected for a further 150min. The same time-course was used for the in vitro study - animals were decapitated 150min after drug administration; the striata were removed and stored at -70°C until analysis. All samples were assayed for dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC-EC. TRH (10µg) increased DA levels in dialysate samples by 190% after two hours (levels of DOPAC and HVA were decreased by 15% and 20% respectively). Tyrosine alone increased DA release by 318%; while its coadministration with TRH (10µg) increased DA release by 450%. A higher dose of TRH (50µg) increased DA release by 780%. All these treatments failed to alter DA metabolism when studied using striatal homogenates. These findings suggest that TRH (or a metabolite) can enhance DA release, and that additional presynaptic amino acid (tyrosine) can potentiate DA release when its level becomes limiting due to sustained nigrostriatal firing. These data also suggest that increased DA release cannot always be predicted by in vitro homogenate studies. (Supported in part by a grant from the Air Force Office of Scientific Research).

482.21

(-)-NALoxONE INCREASES CARBACHOL- EVOKED RELEASE OF DopAMINE, METJENKPHALIN, AND NEUROPEPTIDE Y FROM EX SITU- PERFUSED DOG ADRENAL. M. K. Dousa*, S. L. Christoforlou, D. L. Lucadamo, L. Kash and O. M. Tye. Dept. of Physiology, Mayo Clinic, Rochester, MN and Dept. of Anesthesiology, University of San Diego, La Jolla, CA 92039.

(-)-Naloxone, a stereospecific opioid antagonist, was used to study the modulation of adrenomedullary secretion by endogenous opioid peptides. Experiments were carried out on retrogradely perfused ex situ dog adrenals using 2-min stimulations (S1, S2, S3) by 3×10^-6 M carbachol. Five min before S2, 10^-5 M (-)-naloxone was added to perfusate for the rest of the experiment. In perfusates collected before, during, and after each stimulation, catecholamines (CA) were measured by HPLC with electrochemical detection and [Met]enkephalin (M-E) and neuro­ peptide Y (NPY) by specific radioimmunoassays.

Introduction of 10^-5 M (-)-naloxone into the medium did not change basal levels of CAs and neuro­opeptides; however, met carbachol-evoked release of norepinephrine (NE), epinephrine (E), and dopamine (DA, M-E, and NPY was increased to 138% for NE, 205% for E, 210% for DA, 216% for M-E, and 193% for NPY based on the release during S2 as 100%. No such increase occurred in the presence of (+)-naloxone. The above data showing stereospecificity of (-)-naloxone suggest a probable inhibitory effect of endogenous opioids on adrenomedullary secretion mediated by opioid receptors.

482.22


In digitonin-permeabilized bovine adrenal medullary cells, cis-unsaturated fatty acids (arachidonic acid and oleic acid) enhanced calcium-induced secretion of catecholamines and activation of tyrosine hydroxylase. On the other hand, trans-unsaturated fatty acid (elaidic acid) and saturated fatty acid (stearic acid) had no effect. Catecholamine secretion induced by arachidonic acid was abolished by the removal of ATP and magnesium from the extracellular medium. Phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, enhanced calcium-induced catecholamine secretion. The stimulatory effects of arachidonic acid and PMA were not additive. In soluble fraction of adrenal medullary cells, only cis-unsaturated fatty acids activated protein kinase C in a calcium-dependent manner. These results suggest that cis-unsaturated fatty acids modulate catecholamine secretion and tyrosine hydroxylase activity activation of protein kinase C in adrenal medullary cells.
NORADRENA LNE AXON TERMINALS IN ADULT RAT NEOCORT EX: SYNAP TIC ANTECED AND MICRONVIRONMENT. L. Descaries, F. Seguin, K. Chen, Centre de recherche en neurosciences, Université de Montréal, Montréal, Canada H3C 3J7 and Laboratoire de Neuroimmunologie, I.B.C.N. CNRS, 33077 Bordeaux, France.

The relational characteristics of noradrenergic (NA) axonal varicosities in the upper layers of adult frontal, parietal and occipital cortex were investigated by PAP-electron microscope immunocytochemistry with an antisera against NA-glutathaldehyde-protein conjugate, as well as single thin sections. A large number of varicosities immunostained for TH were scrutinized for the presence of a junctional membrane differentiation and the identity of juxtaposed elements. As a control, random sections were stained with unlabelled varicosities from the same sections and were similarly analyzed. The proportion of synaptic varicosities (incidence) was determined by linear transformation of the relationship between the observed frequency of junctional complexes and the number of thin sections available for examination. Using high or low stringency criteria for the definition of junctional complexes, the synaptic incidence of NA varicosities was thus evaluated at 94 and 76%, respectively, in sharp contrast with that of unlabeled varicosities (88%). The rare NA synapses were axo-dendritic, symmetrical and predominantly found on dendritic shafts versus spines. In each region, the microenvironment of NA varicosities also differed from that of the random population, with a greater number of axon varicosities and smaller number of dendritic spines. The neocortical NA innervation is therefore mostly non-junctional (74-83%) and set in a particular milieu. These data also suggest that the global neuromodulatory roles assigned to the cortical NA input might be subserved by a comparable cellular distribution of NA transduction mechanisms throughout neocortex.

Aim of the present study is to reveal the distributional differences of separate catecholamines in the rat spinal cord. Specific and sensitive antibodies to dopamine (DAi), noradrenaline (NAi) and tyrosine hydroxylase (THi) have been used. Roughly the distributions of DAi-, THi- and NAi-immuno­reactive (DAi, NAi, THi) varicose fibers are comparable. High-density terminal fields are present in the dorsal part of the dorsal horn, in the lateral and central parts of the central grey, the intermediolateral cell column (SII-L2) and on the autonoeural cell clusters in the ventral horn. However, some conspicuous differences are observed: 1) in general DAi and THi terminal fibers are more fine-structured and varicose than NAi fibers. 2) In the dorsal horn DAi fibers are densest in layers I and II, while layer II is nearly devoid; densest NAi fibers are found in layers I and II; a dense population of NAi but not DAi or THi cell bodies is present in layer III (L1-L2), and few NAi neurons are seen in layer I (S1–S2). 3) THi cells are also present in the central spinal (S1–S2) and in the lateral funiculus (SI) dorsolaterally to the sacral (S1). These neurons do not stain for DA or NA. 4) In the ventral funiculus NAi and THi but not DAi descending fiber bundles are present. These results indicate a differential catecholaminergic input to the spinal cord, and the presence of intrinsic spinal NA (L1-L2) and possibly L-SNA (S1–S2) neurons.

IMMUNOCYTOMULTIPLE ANTIGENS IN THE CENTRAL NERVOUS SYSTEM. E.K. Richfield and M. Herkenham. Neurology Deptartment, University of Rochester, Rochester, NY 14605 and Unit on Functional Neuroanatomy, NIMH, Bethesda, MD 20892

The uptake of dopamine (DA), as a mechanism of neurotransmitter inactivation, has been of interest for a variety of reasons. Dysfunction of this complex results in neuropsychiatric disorders, forms the basis of therapy for these disorders and is the site of action of different psycho­pharmacological drugs. Various drugs have been used in ligand binding experiments to study the anatomy and function of this complex. None have proved ideal, with each having different limitations. [3H]-CBR 12935 is a ligand with a number of properties suggesting it might be a good candidate for studying this complex. We have developed a quantitative autoradiographic assay in rodent using [3H]-CBR 12935 that provides several advantages over other assays, including increased sensitivity, specificity, signal-to-noise ratio and reduced cost.

Appropriate buffer studies demonstrated requisite incubation conditions, including a temperature of 2°C, 0.001% ascorbate, and 0.025% albumin. We observed that equilibrium conditions were met and that binding was reversible. In saturation experiments, binding was saturable (Bmax 6.50 pmol/mg protein, Kd was 2.1 nm, and NH was 1.0). Competition experiments revealed binding to two sites, a nonspecific piperazine acceptor site and a specific DA uptake site. In the presence of trans-fluropentol, specific binding was to only the DA uptake complex as determined by the rank order of drugs known to bind to this site. Lesions of the substantia nigra resulted in a 90% decrease in this site in the striatum, whereas striatal kainate lesions did not reduce binding. The anatomical distribution described by this ligand is consistent with other pre- and post-synaptic markers of the DA system.


Prenuclear type I cells of the mammalian carotid body synthesize and release multiple neuroactive agents. Previous pre-embedding, double-labeling immunocytochemical studies indicated that pairs of these neuroactive agents are commonly co-localized in the same cell (Soc. Neurosci. Abst. 44.6, 1988), but failed to reveal subsets of cells with unique patterns of co-occurrence. The present study examined the feasibility of utilizing post-embedding, avidin-biotin immunocytochemistry to co-localize substance P, met-enkephalin, tyrosine hydroxylase, dopamine beta hydroxylase, serotonin, and chromogranin in the same type I cells by means of thin (0.5 µm) serial plastic sections. Individual serial sections of osmicated tissue were deplasticised, treated with 1% periodic acid to remove unspecific binding sites (Chills, J. Soc. Anat. 175: 307, 1986), and immunostained with primary antibody in combination with Vector Elite ABC/HRP reagents. The results indicate that while four of the six markers could be co-localized within the same cell, they occurred in combinations which suggest the existence of functionally distinct subsets of cells. Supported by DEPB Grants NSO7938 and S012636.
484.1


484.2

NEUROTOXICITY: MPTP AND EXCITOTOXIC.

484.3


484.4


484.5


484.6


484.1

NMDDA RECEPTORS MAY BE INVOLVED IN THE TOXIC MECHANISM OF ACTION OF MDMA. K T Finegan*, JJ Skratt*, I J Irwin, and JW Langston. (Spon: J Tetrad) Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

484.2

CELL TYPE-SPECIFIC EXCITATION OF EXCITATORY AMINO ACIDS FOR CULTURED MOUSE AND CHICK RETINAL NEURONS. D. Stenkamp, J. Coyle and R. Adler. Depts. of Neuroscience and Pharmacology, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

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Despite a rapidly accumulating body of scientific literature on the biological effects of MDMA and its metabolites, the mechanisms underlying its mechanism of toxicity are not fully understood. The present study is designed to determine the specific target(s) of toxicity of MDMA in the brain of the chick embryo. Newly fertilized eggs are incubated at 37°C in a humidified atmosphere of 5% CO2 in 95% O2. On the 21st day of gestation (8 days after MPTP is added) the egg is opened, the entire chick brain is removed, rinsed in cold saline, weighed, homogenized in 10 volumes of 0.3% PCA, mixed centrifuged at 20,000 g for 20 min. The extract is assayed for NE, HVA, serotonin (5-HT)- and HIAA, using HPLC with electrochemical detection. In the MPTP group, the egg, a 95% reduction in NE and HVA, and a 70% decrease in HIAA is observed. In the MDMA group, a 50% reduction in NE and HIAA is observed but DA is not significantly reduced.

484.2

The data indicates that MDMA (10 mg/kg) and increasing doses of DEX (7.5 - 45.0 mg/kg/dose) were injected 5 times at 6 hr intervals; combined treatment groups received DEX 20 min before MDMA. Animals were killed 10 days later for assay of SH, DA, and their respective metabolites in the striatum, hippocampus, and cortex. MDMA (10 mg/kg) alone consistently produced a 50 - 70% reduction in SH and DA, while small but significant increases or decreases noted in the striatum or cortex, respectively. These findings suggest that the NMDA receptor-calcium channel complex may be involved in the toxic mechanism of action of MDMA.
484.7

Previous studies from this laboratory have shown that dopaminergic neurons in the substantia nigra were reduced significantly after short term survival following MPTP treatment in mice. In the present studies, young adult male C57BL/6 mice were treated with MPTP over a two day period (total dose 60-90mg/kg i.p.). The animals were allowed to survive for 3 days, 2 months, and up to 4-6 months. After the appropriate survival times, control and MPTP treated mice were anesthetized and perfused intracardially with 4% paraformaldehyde and 1.5% sucrose in 0.1M PO buffer. Serial 10um thick sections were cut through the entire brain and adjacent sections were stained immunocytochemically for tyrosine hydroxylase (TH) and Glial Fibrillary Protein (GFAP). TH-positive neurons in the substantia nigra were quantitated in the control and MPTP-treated mice at different survival times. The number of GFAP stained astrocytes was quantitated in the striatum by sub-dividing it into a medial, lateral, dorsal and ventral compartments. The number of TH-immunoreactive neurons in the substantia nigra (SN) was reduced significantly three days after MPTP treatment. Furthermore, MPTP treatment produced extensive gliosis in the striatum demonstrated by the presence of numerous GFAP-stained astrocytes in these animals compared to the controls. The following will be discussed: (a) regeneration and plasticity of the dopamine neurons in the SN after long term survival following MPTP treatment and (b) astrocyte proliferation in the striatum with long-term survival. Supported by USPHS grant R29 NS24291.

484.9
PROTECTION OF MPTP TOXICITY WITH GANGLIOSIDES IN VIVO AND IN VITRO. F.J. Roisen, J.S. Schwartz* and M. Gupta, Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

Gangliosides are relatively abundant components of the neuronal plasma membrane that stimulate neuronal differentiation in vitro as well as accelerate regeneration in the central nervous system. Ganglioside administration in vivo has been shown to enhance recovery after chemical or physical insult. We have shown that MPTP treatment in young adult mice leads to a decreased number of tyrosine hydroxylase (TH)-positive cell bodies in the substantia nigra (SN). Furthermore, we have also shown that MPTP has a dose-dependent effect on the survival of PC12 cells in vitro. The present studies were undertaken to determine if treatment of mice with gangliosides prior to MPTP injections directly into the brain has any effect on the dopaminergic neurons of the SN, and if pretreatment of PC12 cells with gangliosides and continued treatment with gangliosides in the presence of MPTP has any effect on MPTP toxicity in vitro. Young adult mice were anesthetized and injected stereotaxically with a mixture of bovine brain gangliosides (BBG) into each lateral ventricle (2µl/ventricle, 200µg/ml). After 16-18 hr, half of the control and BBG injected animals were treated with MPTP (Gupta et al., Neurosci. Lett., 1986). Three days later, brains were processed for TH immunocytochemistry and TH neuronal numbers in the SN were quantitated. In vivo studies, PC12 were grown in RPMI 1640 media supplemented with 10% horse serum and 1.5% FBS. Differentiated cells were cultured as above with the addition of 40ng/ml NGF. Neuro-2a cells were maintained in MEM and 10% FBS while C6 cells were cultured in Ham's F10 with 15% horse serum and 2.5% FBS. Cell survival was quantitated microscopically on coded cultures over a 4-6 day period. These studies demonstrate that MPTP has a dose-dependent effect on the survival of PC12 cells irrespective of their level of differentiation. Furthermore, the Neuro-2a and C6 exhibit similar cytotoxicity to MPTP. These results suggest that MPTP toxicity in vitro is not selective to catecholamine cells only since it also affects other cell types. Supported by USPHS grants NS24524 to FJR and NS24291 to MG.

484.10

Numerous studies have shown that the survival of grafted cells in different sites of the CNS in lesioned animals. We have shown previously that MPTP treatment in young adult C57BL/6 mice leads to a decreased number of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra as well as NGF-primed PC12 cells (20,000 cells in 2µl medium/injection site) were transplanted stereotaxically at two sites into the striatum and allowed to survive for up to two weeks. The animals were treated with Cyclosporin-A either prior to or after transplantation to suppress the immune system. Immunocytochemical staining for tyrosine hydroxylase was used to identify the grafted PC12 cells in the striatum. The number of surviving TH-positive transplanted PC12 cells in the striatum for both groups as well as the number of TH-positive neurons in the host substantia nigra was investigated. Mice were given multiple injections of MPTP over a two day period (Gupta et al., Neurosci. Lett., 1986). Three days later, undifferentiated (NGF-naive) as well as NGF-primed PC12 cells (20,000 cells in 2µl medium/injection site) were transplanted stereotaxically at two sites into the striatum and allowed to survive for up to two weeks. The animals were treated with Cyclosporin-A either prior to or after transplantation to suppress the immune system. Immunocytochemical staining for tyrosine hydroxylase was used to identify the grafted PC12 cells in the striatum. The number of surviving TH-positive transplanted PC12 cells in the striatum for both groups as well as the number of TH-positive neurons in the host substantia nigra was investigated. TH-immunoreactive PC12 cells were detected in the majority of the animals. The number of surviving cells ranged from below 100 to over a few thousand per animal. The effect of NGF priming on the survival of transplanted PC12 cells will be presented. Supported by grants NS24524 to MG and NS24291 to MG.

484.11

Several studies have shown that deprenyl treatment prevents MPTP neurotoxicity. The present studies were undertaken to investigate if treatment of MPTP-lesioned mice with the MAO-B inhibitor, deprenyl, has any effect on monoamine oxidase(MAO)-B activity and monoamine levels in different brain regions. Male C57BL/6 mice at 18 months of age were treated with MPTP over a two day period (total dose 60-90mg/kg i.p.). Control animals received vehicle injections. Three days later, half of the control and treated mice were given deprenyl in drinking water (0.035mg/5ml) for 14-18 days. Fresh brains were taken out and several areas including striatum, olfactory tubercle and cortex were dissected, frozen on dry ice and analyzed for MAO activity as well as levels of monoamines and their metabolites. MPTP treatment alone (p<0.01) reduced the levels of dopamin in the striatum and olfactory tubercle and norepinephrine levels in the cortex. Treatment with deprenyl alone or MPTP followed by deprenyl reduced MAO-B activity in all three regions (p<0.01). These studies suggest that deprenyl treatment of MPTP-lesioned animals decreases MAO-B activity thereby preventing further deterioration of monoamine systems in the brain presumably by decreasing free radical formation, but has no effect on amelioration of MPTP toxicity. Supported by USPHS grant R29 NS24291 to MG.
PHARMACOLOGICAL PROFILE OF CGS 18102A, A PROPOSED ANXIOLYTIC WITH 5HT1A AGONIST AND 5HT2 ANTAGONIST PROPERTIES


CGS 18102A, a hexahydrobenzoxazepanopyridine, inhibited 3H-SHT (IC50 = 1.6 mg/kg p.o.), 3H-DPAT (IC50 = 3.1 mg/kg) and 3H-ketanserin (IC50 = 110 nM) binding to the 5HT1A receptor, and 3H-ketanserin (IC50 = 110 nM) binding to the 5HT2A receptor. The compound was inactive at 5HT1B and 5HT2B sites in vitro.

In vivo, CGS 18102A inhibited SHT accumulation in rat cortex (ED50 = 2.7 mg/kg p.o.), indicating 5HT1A agonist effects, but antagonized 5HT-induced head twich (ED50 = 1.6 mg/kg i.p.) in mice, suggesting 5HT2 antagonist properties. The compound did not induce a 5HT-syndrome in rats (30 mg/kg i.p.). CGS 18102A (1-17 mg/kg p.o.) was active in a Cook-Davison model with increases in conflict responding of 25 to 156% above control baseline setting. These results are consistent with other 5HT1A agonists in this model.

This combined 5HT1A agonist and 5HT2 antagonist properties of CGS 18102A may suggest a compound with greater potential for anxiolytic effects than other compounds with only 5HT1A agonist or 5HT2 antagonist properties.


With the reported activity of buspirone as an anxiolytic drug in man, new compounds with 5HT1A agonist and antagonist properties have been tested for anxiolytic activity in various animal models. We have studied these compounds in the exploratory light/dark test (Costall et al., J. Pharm. Pharmac., 40: 309, 1988), the social interaction test (IC50 = 3.16-3.16 mg/kg p.o. and i.p. and ip-sapirone (18.7-3.16 mg/kg) increased the time the mice spent in the light area, from a vehicle control value of 31% to as much as 72% and 53%, respectively. The alleged pure 5HT1A agonist, B-OH-DPAT, was active in doses of 0.001 to 3.16 mg/kg.

NAN-190, a putative 5-HT1A agonist (Glennon et al., Eur. J. Pharmac., 154: 339, 1988), was inactive in increasing time in the light area in doses up to 0.1 mg/kg IP. Higher doses did not produce a consistent serotonin syndrome. Furthermore, NAN-190 did not potentiate the hypertensive effect of B-OH-DPAT (2.5 mg/kg IP). 5.6 mg/kg B-OH-DPAT antagonized the hypertensive effect of B-OH-DPAT. Thus, although the hypertensive effect of NAN-190 was not due to a 5-HT1A agonistic action, the blockade of B-OH-DPAT hypothesis suggest that NAN-190 is a 5-HT1A antagonist. The data presented here suggest that 5-HT1A agonists, but not 5-HT1A antagonists, may possess anxiolytic activity.

THE PHARMACOLOGICAL PROFILES OF 5-HT1A RELATED ANXIOLYTICS: EFFECTS ON FIRING RATES OF NORADRENALINE CELLS RELATED TO EFFECTS ON Dopamine CELLS THAN TO EFFECTS ON 5-HT CELLS. J.T. Lum and M.T. Piertcey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

3H-SM-3997 binding sites were more selectively inhibited by that of Bus or Ipsi, and is stronger than that of Gep. It was also suggested that 1-PP does not participate in the anxiolytic action of these anxiolytics.

NEUROPHARMACOLOGY OF 5-HT1A AGONISTS: EFFECTS ON FIRING RATES OF NOREPINEPHRINE CELLS MORE RELATED TO EFFECTS ON Dopamine CELLS THAN TO EFFECTS ON 5-HT CELLS. J.T. Lum and M.T. Piertcey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

485.1


The forced swim test has been used as an animal model to screen antidepressant drug activity; single injections of certain antidepressant drugs will reverse the learned helplessness effect evident in untreated animals. We report here that, additionally, there is an anxiogenic component to this test; treatment of male Long-Evans rats with 5 or 10 mg/kg 1-PP 21 days prior to the training session of the forced swim test with the anxiogenic drug, diazepam (2 mg/kg i.p.), resulted in significantly decreased immobility time in the forced swim test when compared to a vehicle-treated control group. Neither 3 nor 21 day diazepam-treated groups differed on the retest from groups of rats treated for the same time periods with the clasically anxiogenic drug, methysergide (10 mg/kg i.p.). Interestingly, on the training session day, 3 day treatment with diazepam alone decreased the immobility latency during the first 5 mins of the training session, whereas 21 day treatment of both diazepam or amitriptyline resulted in decreased immobility latencies during this first 5 minute period. This study suggests that a pharmacokinetic component of the forced swim test is significant and that biochemical changes attributed to effects in depression with this model should be made with caution.
485.7


U-67413B, a diphydorphenalen-4-ol (2-dipropylamine-2,3-dihydro-monohydropyridine), was found to be a 5-HT agonist with dopaminergic antagonist properties. Other standard 5-HT 1A agonists, in contrast, are with varying selectivity (J.T. Lum and M.F. Piercey, this meeting). U-67413B displaced 3H-D-OH-DPAT from 5-HT1A binding sites with a potency of 10-30 mg/kg s.c. displayed the typical 5-HT1a syndrome in rats (forepaw tawing, flattening of posture) with no evidence of dopaminergic activity. Hypothermia in mice had an ED50 of 2.3 mg/kg s.c., less than that for buspironone (3.1), but more than that for gepirone (1.3), ipsapirone (1.3), and 8-OH-DPAT (0.13). Similarly, U-67413B was less potent than standard agonists in depressing 5-HT neuron firing rates in rat dorsal raphe (ED50=50 ug/kg i.v. vs. 1.6-15 ug/kg). On dopamine cells, U-67413B partially depressed firing rates (ED50=50 ug/kg). Using HPLC, 10 mg/kg s.c. U-67413B decreased 5-HTP, 5-HT, 5-HIAA, GABA, GABA, and HVA. Diazepam and U-67413B displayed anxiolytic activity on the mouse 4-plate test, but buspironone did not.

485.8

BUSPIRONE: EFFECTS ON SEROTONIN/NOREPINEPHRINE NERVE IMPULSE FREQUENCY AND RELEASE MECHANISMS. P.A. Broderick and F.T. Pfeffer*, J.T. Lum, W.E. Hoffmann, and M.F. Piercey. Pharmacology Dept., CURY Medical School, Convent Ave. and 138th St., NY, NY 10031, and CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

Buspirone (Upjohn Company, Kalamazoo, MI 49001) and 5-HT systems have been implicated as being anxiogenic. Both are depressed by benzodiazepine anxiolytics. We have used micelle electrode recordings of nerve cell impulse frequency and in vivo voltammetry recordings of hippocampal 5-HT (Neuropeptides 10:369, 1987) and NE (Neurosci. Lett. 95:275, 1988) release to evaluate the effects of choices of the 5-HT1A anxiolytic buspirone on rat 5-HT and NE neurons. As previously reported, (Eur. J. Pharmacol. 149:9, 1988) buspirone depressed 5-HT neuron firing rates in dorsal raphe with a potency of 15 ug/kg i.v. It increased NE neuron firing rates in locus coeruleus with a dose-response curve virtually congruent with that for 5-HT neuron depression. In hippocampus, 1 mg/kg s.c. buspironone decreased 5-HT release moderately, but dramatically decreased NE release. It is concluded that, like benzodiazepine, buspironone depresses both NE and 5-HT systems and that the increase in NE neuron firing rates is a response to negative feedback control.

485.9

ANTAGONISM OF SHT CELL BODY AUTOCEPTORS (5HTIA SUBTYPE) BY THE DOPAMINE AUTOCEPTOR ANTAGONIST (+)-AJ 76. M.F. piercey, J.T. Lum and W.E. Hoffmann. CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

The dopamine autoreceptor antagonist (+)-AJ 76 (Svensson et al., 1986, Arch. Pharmacol., 333:234 and Hoffmann et al., 1986, Neurosci. Abs. 14:524) has been evaluated for its effects on firing rates of rat 5-HT neurons in dorsal raphe. (+)-AJ 76 weakly depressed firing rates in untreated animals. Starting with a threshold dose of 100 ug/kg i.v., there was a shallow dose-response curve which peaked at about 50% depression with 3000 ug/kg of drug. In animals pretreated with 10 mg/kg (+)-AJ 76, the ED50 for the potent 5-HT1A agonist 8-OH-DPAT was 48 ug/kg compared to only 1.3 ug/kg in untreated animals. It is concluded that (+)-AJ 76 is a partial agonist at the 5-HT1A autoreceptor. (+)-AJ 76 also antagonized the hypothermic effects of 5-HT1A in mice, an effect produced by postsynaptic 5-HT1A receptor activation. A dose of 30 mg/kg s.c. shifted the ED50 for 8-OH-DPAT from 0.23 mg/kg to 1.73 mg/kg, but did not at all affect body temperature itself. (+)-AJ 76 had little effect on norepinephrine cell firing rates. It is concluded that (+)-AJ 76 is a 5-HT1A receptor antagonist with weak agonist intrinsic activity that is observable only in the presence of large numbers of spare receptors (e.g. dorsal raphe).

485.10

2-DEOXYGLOSE AUTORADIOGRAPHY PINPOINTS SEROTONIN NEURON DEPRESSION AS A COMMON MODE OF ACTION FOR 5-HT1A AND BENZODIAZEPINE-TYPE ANXIOLYTICS. W.E. Hoffmann and M.F. Piercey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

2-Deoxyglucose autoradiography in rats was used to compare regional metabolic effects of 5-HT1A anxiolytics (buspirone, U-67413B), to those for alprazolam, a benzodiazepine anxiolytic, and FG 7142, an anxiogenic benzodiazepine inverse agonist. U-67413B was chosen because, unlike buspirone, it totally lacks dopamine antagonism and does not excite locus coeruleus neurons. Buspirone, 1 or 10 mg/kg i.p., and U-67413B, 10 mg/kg i.p., depressed metabolism in 35 of 71 brain areas. Thirty-five regions were depressed by alprazolam, 3 mg/kg i.p. and/or stimulated by FG 7142, 1 or 20 mg/kg i.p. Regions affected by each 5-HT1A agonist were highly correlated with each other, but not with those affected by the benzodiazepine receptor ligands. 5-HT1A agonists depressed metabolism in 5-HT cell body (dorsal and median raphe, interpeduncular n.) and projection areas. The hippocampus and wide areas of the cerebral cortex (cingulate, entorhinal, parietal, visual and motor) were all depressed. All four drugs affected 5-HT cell body areas (interpeduncular n., median raphe) and the cingulate and retrosplenial cerebral cortices. The results are consistent with 5-HT neuron depression as a common site for benzodiazepine and non-benzodiazepine anxiolytics.

485.11

EFFECT OF THREE BENZODIAZEPINES ON MONGOLIAN GERBIL ACTIVITY. K.C. WOLFP, A.E. HARRIMAN*, AND S. SANGHAI. Lab of Comparative Psychology, OK State Univ. Dept. of Psych, Stillwater, OK 74079.

Four male and four female gerbils were deprived of food 24 hrs before intraperitoneal injections of normal Saline 0.1 cc, 0.3 cc, 0.6 cc; Flurazepam 30 mg/kg, 45 mg/kg, 60 mg/kg; Temazepam 0.75 mg/kg, 1.5 mg/kg, and Triazolam 0.0125 mg/kg, 0.025 mg/kg, 0.05 mg/kg. In addition, sham injections were also used. The administration of drug and dosage was conducted and analyzed using a factorial repeated measures design. Results suggest that all dosage levels of Flurazepam and Temazepam increase bar pressing as measured in the Skinner Box using a schedule of continuous-reinforcement. On the other hand, all dosage levels of Triazolam decreased the rate of bar pressing.

The second part of the study involved nine male gerbils, each receiving the same drug and dosage level used in the first part of the experiment. The analysis was also a factorial repeated measures design. The animals were tested using an activity wheel and then micros were placed in an open field situation. Results indicated that those gerbils receiving Flurazepam and Temazepam had an increase in motor activity as well as grooming, marking, rearing, and leaping behavior. The opposite was found for those gerbils receiving Triazolam. We also found an increase in seizure activity among those gerbils receiving 45 mg/kg of Flurazepam and 1.5 mg/kg of Temazepam.

485.12


The late exploratory test was developed in mice to predict activity of new hypnotics in humans.

Pairs of male CF-1 mice were placed into 8” X 8” Omnitech Digiscan chambers under room light immediately after i.p. injection of triazolam, flurazepam, diazepam, zopiclone, zolpidem, diphenhydramine, Na phenobarbital, RO 16-6028, chloral hydrate (oral), or vehicle. After 15 min, when the mice were partially habituated to the chamber, locomotor activity (total distance) was recorded automatically for the next 5 min. By linear regression for these I 0 standards, the minimum effective dose (m.e.d. for triazolam) was 0.004 mg/kg.

In conclusion, the late exploratory test predicts human hypnotic activity of compounds.

SIGMA receptors in brain are defined as the high affinity sites that bind \(\text{[^3H]3-PPP}\) selectively and competitively inhibit sigma binding but are not displaced by PCP or dopamine binding \(\text{(Purifoy et al., 1986; Ferris et al., 1986)}\). Sertraline is a new antidepressant and selective \(\text{5HT}\) receptor antagonist; e.g., r-

**485.17**


Sigma receptors in brain are defined as the high affinity binding sites labeled by \(\text{[^3H]3-PPP}\) (NAN: SKFF 10.047), (S)-\(\text{[^3H]3-PPP}\) and (S)-\(\text{[^3H]3-PPP}\) are also radioisotopes of sigma binding sites. Affinity for \(\text{[^3H]3-PPP}\) receptors has been invoked to account for an antipsychotic effect, (as predicted by animal behavioral tests) of agents that are not dopamine receptor antagonists; e.g., rizamala (Ferris et al., 1986) and BMY 14802 stereoselectively inhibits sigma binding, and we have shown that this agent selectively and competitively inhibits sigma binding but not PCP or dopamine binding \(\text{(Oh, ]].en. 1986; Ferris et al., 1986; Lehel et al., 1987)}\). Sertraline, a new antidepressant and selective \(\text{5HT}\) uptake blocker (Ko et al., 1983), was found to be a potent inhibitor of \(\text{[^3H]3-PPP}\) binding to brain membranes \(\text{(EC_7.7 M)}\). Sertraline's high affinity for \(\text{[^3H]3-PPP}\) receptors was dependent on its conformation as well as on the CI atoms in its pendant phenyl ring. Efficacy of binding to \(\text{[^3H]3-PPP}\) in brain in vivo was determined by labeling the comparison of brain \(\text{[^3H]3-PPP}\) binding in a variety of experimental conditions and inhibition of \(\text{[^3H]3-PPP}\) binding to \(\text{[^3H]3-PPP}\) receptors in mouse brain was then assessed as that for blocking \(\text{[^3H]3-PPP}\) uptake in rat brain \(\text{(EC_50 0.7 mol/kg i.p.})\). 

**485.15**

**IS DEXTROMETHORPHAN A SIGMA ANTAGONIST? INTERACTIONS WITH \(\text{[^3H]SKF10047}\). F. C. Tortella and L. Rebes*, Neuropharmacology, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.**

The non-opioid antitussive Dextromethorphan (DM) and the sigma ligand \(\text{[^3H]SKF10047}\) appear to share a common binding site \(\text{(rSKF)}\) of similar potency in brain as well as in behavioral paradigms. DM antagonizes these "psychotomimetic" effects of \(\text{[^3H]SKF10047}\). The attenuated, and the \(\text{[^3H]SKF10047}\)-induced increase in EEG spectral power in the 1-5 and 7.5-10 Hz range and decreases in complexity and edge frequency. In contrast, \(\text{[^3H]SKF10047}\) produces a complex continuum of EEG and behavioral changes independent of its conformation as well as on the CI atoms in its pendant phenyl ring. Efficacy of binding to \(\text{[^3H]3-PPP}\) in brain in vivo was determined by labeling the comparison of brain \(\text{[^3H]3-PPP}\) binding in a variety of experimental conditions and inhibition of \(\text{[^3H]3-PPP}\) binding to \(\text{[^3H]3-PPP}\) receptors in mouse brain was then assessed as that for blocking \(\text{[^3H]3-PPP}\) uptake in rat brain \(\text{(EC_50 0.7 mol/kg i.p.})\). 

**485.16**

**ANTAGONISM OF A MODEL SIGMA SYNDROME.** Edgar T. Iwamoto, Dept. of Pharmacology, College of Medicine, Univ. of Kentucky, Lexington, KY 40536

We have presented evidence for a drug-induced activation of central sigma systems in rats \(\text{(Life Sci. 44:1547, 1989)}\) in which motor behavior is characterized by an initial 20 min period of retropulsion (RETRO) and sideways-circling (SIDE) followed by 50 to 100 min of a more consistent syndrome initiated by a subcutaneous (SC) injection of \(\text{[^3H]3-PPP}\) binding to rat brain membranes \(\text{(ID_50 500 nM)}\). Sertraline, a new antidepressant and selective \(\text{5HT}\) receptor antagonist; e.g., r-

**485.14**

**BUSPIRONE, GEPIRONE, IPSAPRINE, AND \(\text{[^3H]3-PPP}\): COMPARISON OF TWO MOUSE AGGRESSION MODELS.** J. A. Ostvneen* and P. J. Schreur, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Four putative \(\text{5HTA}\) anxiolytics were tested for their effects on aggression elicited by footshock or by chronic isolation. In the footshock model, pairs of male \(\text{CF-1 mice (28-30 g) were confined in a small space and shocked via the floor grid (20 sec maximum) until dominance was established. Pairs of dominant mice were injected with drug i.p. 30 min before receiving intermittent shocks (maximum 5 shocks) once were separated as soon as fighting began and the number of shocks was recorded. For isolation aggression, male \(\text{CF-1 mice were housed 4 or 5 per group 4 or 1 time a week. An isolated "resident" mouse was injected with drug 30 min before an untreated, group-housed "intruder" was introduced. The intruder was removed as soon as fighting began and the number of seconds in which no fighting was recorded. Buspirone HCI was active at 3-30 mg/kg in the isolation model but inactive in footshock to 30 mg/kg. For isolation and footshock models, respectively, 8-OH-DPAT HBr [8-(hydroxy-dipropylaminotetralin HBr] was active at 1-10 mg/kg and 3-10 mg/kg, gepirone HCl at 3-30 mg/kg and 10-30 mg/kg, and ipsapirone HCl at 10-30 mg/kg and 30 mg/kg. Isolation-induced aggression is a more sensitive test than footshock aggression for these \(\text{5HTA}\) anxiolytics.**

**485.18**

**STEREOSELECTIVE RECEPTOR BINDING AND BIOLOGICAL ACTION OF THE POTENTIAL SIGMA ANTIPSYCHOTIC BMY 14802.** Duncan P. Taylor, Susan R. Behling*, Jennifer Delbevv*, and Michael S. Eleon. CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660.

BMY 14802 has been identified as a potential antipsychotic agent in behavioral testing in the Neurogator. \text{[^3H]3-PPP}\) Binding 32 140

**Conditioned Avoidance** 22 39

**Apomorphine Stereotypy** 44 (38-50) 25 (17-37)

**Catalepsy Reversal** 11 (7-19) 38 (27-34)

\(\text{[^3H]3-PPP}\) Binding 32 140

**Conditioned Avoidance** 22 39

**Apomorphine Stereotypy** 44 (38-50) 25 (17-37)

**Catalepsy Reversal** 11 (7-19) 38 (27-34)

Data are \(\text{K(J) (mM)}\) or \(\text{ED_{50} (mg/kg, p.o. with 95% fiducial limits in parentheses for tests in rats.}^{\text{[485.18}}\)
POST-JUNCTIONAL INHIBITION BY SIGMA AND PCP LIGANDS OF RAT TAIL ARTERY CONTRACTILE RESPONSES: T. Massam* and S.P. Dackis. (SPON: J. O'NO) Department of Pharmacology, College of Medicine, University of California, Irvine 92617.

(3)PPP (3-[3-hydroxyphenyl]-N-[1-propyl]-piperidine) acts on both dopamine and sigma receptors in the perfused rat tail artery in vitro confirming previous reports. We also demonstrate an additional mechanism of action of (+)3PPP; (+)SKF 10047, (+)-n-propyl-N-normetanephrine (nPT), and (+)(+)-Ritalin (Ri) displaced [(3H)-TCP] from the noradrenaline uptake system. The inhibition of noradrenaline uptake by these agents was revealed as a potentiation of the contractile response to noradrenaline or transmural nerve stimulation, an effect which was blocked by cocaine, but not desmethylcortone (DOC). In the presence of noradrenaline uptake blockers (cocaine and DOC), a third action of (+)3PPP and (+) SKF 10047 was unveiled, an inhibition of the contractile response to noradrenaline, possibly via sigma receptors. The order of potency of additional sigma ligands, which also inhibit contractible responses to noradrenaline, haloperidol, DTG, 1,3-di-octylglycine-guanidines: BW 214f, cis-9,13-[1,5-dimethyl-1-piperazinyl]propyl]carbazole dihydrochloride (rimcacoze), and BMI 14802, (4-fluoro-2-pyrimidinyl)-1-piperazinyl butanoate suggests that sigma receptors may be present in blood vessels and could play a role in modulating contractile responses.

Supported by NIH grant #DK63629 and Fellowship #DA05539-02

5-HT FUNCTION IN THE BIOCHEMICAL AND BEHAVIORAL RESPONSES TO MCPP IN HEALTHY SUBJECTS AND SCHIZOPHRENICS. J.P. Selby*, J.H. Krystal, L.N. Price, S.W. Woods, G.R.

Hannigler, D. Grant, Yale University School of Medicine, West Haven VANC, West Haven, CT 06516.

Research with 5-HT2 agonists have been limited by hallucinogenic properties. 4-phenylphosphinylpiperazine (MCP Kap) acts at both 5-HT1 and 5-HT2 receptors in animals. We evaluated the ritanserin (5HT2 antagonist)-reversible MCPP, 3) PLA-PLA, 4) Ritanserin-PLA. SCHIZ's (n=5) completed MCPP and PLA test days. RESULTS: Ritanserin attenuated MCPP-induced increases in prolactin, growth hormone, cortisol, drowsiness, feeling high, and anxiety. SCHIZ's (n=5) completed MCPP and PLA test days. Ritanserin attenuated MCPP-induced increases in prolactin, growth hormone, cortisol, drowsiness, feeling high, and anxiety. Ritanserin also produced a significant MCPP-Induced increases in prolactin, growth hormone, cortisol, drowsiness, feeling high, and anxiety.

5-HT Function in the Psychokinesis Tests in Healthy Subjects. Supported by VA Project #103.


The ability of compounds to interact with sites labeled by sigma receptor ligands was examined in whole guinea pig brain membranes. Haloperidol exhibited equal affinity (Ki = 4-6 nM) for sites labeled by [3H] DTG and [3H] (+)-3-PPP, while DTG was 2-fold more potent against [3H] MCPP (DTG Ki = 5.5 nM), and (+)-3-PPP was 3-fold more potent against [3H] (+)-3-PPP (Ki = 36 nM). In contrast, dextromethorphan (DM) was 20-fold more potent against [3H] (+)-3-PPP (Ki = 0.12 µM) than [3H] DTG (Ki = 2.6 µM). Likewise, caramiphene displaced [3H] (+)-3-PPP with a potency (Ki = 9-9 M) 10-fold greater than observed for [3H] DTG (Ki = 92 nM). DM also interacted with sigma receptor binding sites labeled with [3H] haloperidol and [3H] SKF-10.047, exhibiting a potency vs [3H] haloperidol (Ki = 8-3 µM) similar to that observed vs [3H] DTG, and a Ki of 0.18 µM against [3H] SKF-10.047, similar to that observed vs [3H] (+)-3-PPP.

Phencyclidine (300 µM), which is known to enhance [3H] (+)-3-PPP binding, also enhanced [3H] SKF-10.047 binding but did not increase the binding of [3H] DTG or [3H] haloperidol. In addition, phencyclidine enhanced the potency of DM to displace both [3H] DTG and [3H] (+)-3-PPP.

These findings suggest that sigma receptor ligands bind to either distinct sites, or different configurations of the same site which are distinguished by DM but not by haloperidol.

The quantitative assessment of grip strength (GS) in rodents is used by neurotoxologists as one means of evaluating potential neurotoxic effects of environmental and pharmaceutical agents. Various forms of GS assessment have also been used in the specific evaluation of the muscle relaxant properties of drugs. The current study explored the effects of several muscle relaxants (e.g., diazepam, midazolam, buprenorphine, and midtrendal) on GS in cats. An example of this paradigm includes the use of a carrier procedure by Meyer et al. (Neurobehav. Toxicol. 1:233-6, 1979). This procedure utilizes a strain gauge to measure the lateral pull of force, e.g., exerted by the cat's forelimb, as an index of muscle relaxation. Other drugs such as anesthetics, major tranquilizers, stimulants, etc. were also tested in order to determine the specificity of the effects of the muscle relaxants in this paradigm. The muscle relaxants diazepam, midazolam, buprenorphine, and midtrendal dose-dependently reduced forelimb GS. Z-Amino-7-phosphonohexanoic acid, which has been shown to have muscle relaxant effects, also reduced GS (Pentobarbital), ethanol (i.e., phencyclidine, ketamine and chlorpromazine) also reduced GS but at behaviorally-imparing doses. Lithium chloride, at doses typically used to induce taste aversions, and clonidine, at doses that reduce locomotor activity, did not affect GS. In addition, stimulant doses of amphetamine and caffeine, but not of morphine, increased GS in a dose-dependent manner. These results extend the findings of Meyer et al., and suggest that this forelimb GS procedure may be useful for the evaluation of the muscle relaxant properties of drugs.


The magnetic evoked field (MEF) is due to currents directly associated with active neurons plus those due to inhomogeneity in electrical conductivity of the tissue. We studied the conditions under which tissue inhomogeneity may significantly modify the MEF due to neuronal currents, utilizing the isolated cerebellar preparation of turtle (Okada et al., Brain Res. 421:151, 1987). The extracellular conductivity ($\sigma_e$) of this lissencephalic, oblate neuronal current to be enhanced by a factor of 2.3 by the cerebellar surface boundary and reduced by a factor of 1.5 by the conductivity change from the molecular to the granular layers. These large effects arise from the proximity of neuronal sources to the boundaries (0.5 mm). The conductivity boundaries primarily affect magnitude of the MEF, inasmuch as neuronal source locations are stationary in time, but it could also modify the temporal waveform if the source location moves in depth as in the case of cortical sources. Supported by NINDS grant NS21149.


The midlatency (20-30 msec) positive auditory evoked potential wave A in the cat appears to be a correlate of P1 in humans. Both potentials are absent at rapid click repetition rates (10/sec) and during slow-wave sleep but present during wakefulness and REM sleep. We previously showed that wave A is eliminated by bilateral lesions of the pedunculopontine tegmental nucleus in the midbrain reticular formation. This nucleus contains cholinergic cells, and we have also shown that scopolamine, a muscarinic cholinergic antagonist, eliminates wave A. To test further the hypothesis that wave A is dependent on cholinergic cells, we studied the effects of cholinergic agonists. EEG was recorded with an amplifier filter setting at 10 Hz-3 kHz, from awake cats, with clicks presented at a rate of 0.2/s. In all cats tested, scopolamine (0.3 mg/kg, subcutaneous) eliminated wave A. Both carbachol (0.05 mg/kg, subcutaneous), a muscarinic agonist, and physostigmine (0.25 mg/kg, intravenous), a cholinesterase inhibitor, reversed this scopolamine effect, i.e., the eliminated wave A returned. Control intravenous injections of the same volume (2 cc) of carrier did not have these effects. Nicotine bitartrate (0.1 mg/kg, intravenous) had no effect on wave A. These results extend prior work, all of which supports the hypothesis that wave A is dependent on muscarinic cholinergic activity. (Supported by USPHS Grants HD05958 and NS25400).


The human MLRs consist of a 30-40 msec positivity, "P1", a 40-50 msec negativity, and a subsequent 50-65 msec positivity, "P2". The human P1 and the cat MLR, "wave A", both disappear as click rates exceed 1/sec and during slow-wave sleep; both increase during REM sleep to equal their amplitudes during wakefulness. Additional data in the cat support the hypothesis that the human P1 and cat wave A are generated by cholinergic cells within the ascending reticular arousal system (RAS) projecting to cholinoreceptive target cells in the thalamus, intero as forebrain cholinergic dysfunction occurs in Alzheimer's disease (AD), we postulated that brastem cholinergic dysfunction might likewise occur and be reflected by an abnormal P1. In order to test this prediction, the following study was carried out on 6 AD men, mean age 63 years. Probable AD (N=2) was diagnosed using NINCDS-ADRDA guidelines. Definite AD (N=4) was diagnosed following cortical biopsy which demonstrated confirmatory plaques and neurofibrori tangles. An age-matched group of healthy, neuropsychiatrically normal men served as controls. Click stimuli were presented binaurally at an intensity 55 dB above hearing threshold for each subject. MLRs were recorded from a midline scalp electrode referenced to linked mastoid sites with filter bandwidths of 10-300 Hz. Comparisons between AD and age-matched control groups indicated normal auditory brainstem and P2 responses. In contrast, P1 was missing or dramatically reduced in the AD subjects. While the present sample size is small, this first demonstration of significant P1 reduction in AD suggests RAS dysfunction involving cholinergic cells of the brainstem.
A LEFT HEMISPHERIC DEFICIT IN P200 FROM ALZHEIMER'S PATIENTS RECORDED IN A VISUAL MEMORY TASK. S. Sands, J. De La Chapa, and S. Smith, Dept. of Psychology, Univ. Texas at El Paso, El Paso, TX, 79968.

A voltage deficit maximal in the left hemisphere was observed in Alzheimer's Disease (AD) patients performing a serial probe recognition (SPR) task compared to age match controls. Six suspected Alzheimer's patients meeting NINCDS-ADRDA selection criteria were selected from a larger population of dementia patients. They were compared to 15 controls free of significant neurological or cognitive impairing disorders. Event-related potentials (ERPs) were recorded from 28 electrode sites (modified international10-20 system) while subjects performed a variable list length (1,4 and 8 item) SPR task. Ocular artifact was monitored via VEOG and HEOG leads. ERPs were averaged separately for list and probe items. A reduction in P200 amplitude was observed in left-hemispheric recording sites (i.e., F3,C3,T3,T5) from the AD patients. This effect was not observed in controls subjects who did poorer in the memory task. These results are consistent with recent PET findings of increased left-hemispheric abnormalities in suspected AD.


The motor evoked potential (MEP) is a new neurological technique to stimulate or evoke potentials which can be monitored along the spinal cord in clinical use, the MEP has been shown to be far more reliable than the somatosensory evoked potentials for predicting functional recovery after spinal cord injury. The goal of this study was to develop a technique for monitoring MEPs chronically in the spinal cord of rats, and to test if the MEP in chronically recording sites had functional significance.

Two pairs of teflon coated stainless steel wire electrodes with an exposed tip of 1 mm were chronically implanted on the dural surface at T6 and LI. Wires were guided between the two recording electrodes did not change recording sites. The improvement of the L/T ratio was correlated with the recovery of hindlimb locomotion.

COVARIANCE AMONG MOTOR EVENTS IN SCHIZOPHRENIA: SMOOTH PURSUIT EYE MOVEMENTS, SPINAL REFLEXES AND REACTION TIME. R.T. Pivar, F.W. Syman*, and P.M. Cooper*, Dept. of Psychiatry, Univ. of Ottawa and Ottawa General Hospital, Ottawa, Ontario, K1H 8L6.

The present study examined the extent to which motor behaviors, shown to be deviant in separate populations of schizophrenics, would covary in the same patients. Motor activities studied included smooth pursuit eye tracking, variations in spinal reflexes and reaction time (RT). The amplitude of the spinal monosynaptic H-reflex, and button-press reaction time (RT) to an auditory tone. These measures were assessed in: actively-ill schizophrenics (n=17); schizophrenics in remission (n=12), and normals (n=17). Diagnosis and clinical status were determined independently by two psychiatrists using DSM-III criteria, interviews and hospital records. Measures were recorded using standardized techniques, electronically processed and analyzed using analysis of variance procedures. For all measures, actively-ill patients deviated from comparison groups, demonstrating enhanced reflex excitability, impaired tracking and slow RTs. With the exception of the RT comparison with remitted patients, these differences were statistically significant. The results indicate deviant responses at several levels of motor functioning in actively-ill schizophrenics. Furthermore, since the degree of abnormality abates considerably in remitted schizophrenics, to a great extent these effects appear to be state dependent.

Research assisted by the Ontario Mental Health Foundation.


The cerebellar evoked potential (CEP) has been proposed as a clinical tool to assess the integrity of the extrapyramidal system. Electrical stimulation of the cerebellar cortex produces a response traveling in the ventral cord. However, the pathways producing these potentials have to be evaluated. The purpose of this study was to characterize the components of the CEP in the rat and to clarify the pathways. The rat was placed in a stereotaxic head holder and cranial window was performed to expose cerebellar cortex. Two laminectomies were performed to expose exposed SM cortex, which has been proven to be the most significant area on the spinal cord. Epidural recordings were made with a pair of teflon insulated, platinum wire electrodes with 1 mm exposed tips. The cerebellum was stimulated by an electrical stimulus to the dorsal root. The recordings were made at the occipital bone on top of the intermediate zone in lobule 6. The CEPs recorded on the thalamic cord consisted of several positive peaks with conduction velocities of 30-100 m/sec. The upper CEPs recorded on the lumbar cord showed only two positive peaks with conduction velocities of 23m/sec and 45m/sec. Dorsal hemissection of the spinal cord between the two recording electrodes did not change the components of the CEP monitored at T6 or L1.

The cerebellar cortex is most affected in Late Infantile cases. These relationships are being extended in serial recording of ERGs and VEPs, and in our expanding data base.
486.11
DIABETIC ENCEPHALOPATHY: CAN IT BE PREDICTED FROM THE RETINAL STATUS? M.A. Kabene1, J. Everett2, C. Harnois2
(SPOK: Harold W. Gordon), School of Psychology2, and Department of Ophthalmology and Vision Science 1, University of British Columbia, Canada.

Different complications are related to diabetes, and among them is retinopathy. Few things are known about the possible effects of diabetes on other brain systems. We are currently investigating retinopathy and diabetes using fMRI and MEG.

486.12
ENHANCEMENT OF CORTICAL EVOKED POTENTIALS BY ETOTOIDATE. LOCUS AND POSSIBLE MECHANISM. S.K. Samra and L.S. Sorkin, Depts. of Anesthesiology, Anat. and Neuroscy., Univ. of Texas Med. Branch at Galveston, Galveston, TX 77550.

In contrast with all clinically used anesthetics, which are known to decrease the amplitude (ampl) of cortical evoked potentials (CEP), etomodate has been shown to enhance CEP in humans. This study attempts to define the locus and possible mechanism of this phenomenon.

Cats were anesthetized with halothane (0.5-1.5%) in a mixture of 50% N2O in oxygen. Blood pressure (BP) was continuously monitored. Core temperature and end-tidal CO2 were maintained within physiological limits. Evoked potentials were recorded from VPL thalamus and either EI or II sensory cortex following tibial nerve stimulation.

Etomodate (1-3 mg/kg) caused a transient, dose-dependent decrease in BP which was accompanied by a decrease in ampl of CEP (both N1 and P1). Return to normal BP was accompanied by a 50-150% increase in ampl of CEP compared to control tracings. Pretreatment with GABA antagonists and agonists modified the etomodate enhancement of CEP. Thalamic recordings remained unchanged throughout. These results suggest that enhancement of CEP is occurring within the cerebral cortex and involves GABA receptors. (Supported by NIH grant NS 11255).

486.13
AUDITORY LONG LATENCY ERPs IN RATS: REGIONAL AND NEUROCHEMICAL FINDINGS. C. Ehrer, F. Wall, R. Chaplin2. Department of La Jolla, R.I.S.C., La Jolla, CA 92037

Animal models of event related potentials (ERPs) have recently been developed in order to gain further understanding of the physiological variables which underlie these brain potentials. The present study utilized unanesthetized rats in order to: 1) evaluate whether a P3-like component could be identified in this animal in response to variations in the stimulus characteristics of an auditory oddball paradigm; 2) compare ERP's from different brain sites; 3) test the effects of amine depletion on ERP morphology. Sixty-one male Sprague-Dawley rats used in the above studies. The results of these studies showed that all electrode sites tested (corpus, nucleus accumbens, amygdala, dorsal hippocampus, locus coeruleus) contain large amplitude potentials in the 50-100 msec latency range which are sensitive to changes in stimulus characteristics such as probability and loudness. Whereas late positivities in the 300-400 msec latency range were only identified in the dorsal hippocampus and in the amygdala. Destruction of the dopamine containing neurons of the VTA using 6-OHDA was not found to produce any changes in ERP responses or of any of the components in the lesioned rats as compared to the sham animals. Whereas the serotonin depletion produced by PCPA injections was found to produce significant reductions in the amplitude of the N1-like component in cortex. Dorsal noradrenergic bundle lesions produced by 6-OHDA also caused significant changes in ERP components. Lesioned animals were found to have increases in amplitude of the N1-like potentials in response to frequent tones in cortical leads and decreases in the amplitude of the P3-like potential in hippocampal leads in response to infrequent tones. This finding is consistent with a role for NE in the hippocampus in the processing of novel or selective stimuli. These studies suggest that the rat may be a good model for future exploration of long-latency ERPs. (Supported by NIAAA 00098, 06059).

486.15
CHANGES IN HUMAN CORTICAL MOTOR REPRESENTATION AREAS OF MUSCLES PROXIMAL TO THE STUMP AFTER AMPUTATIONS: A STUDY WITH TRANSCRANIAL MAGNETIC STIMULATION. L. Cohen and M. Hallett. Human Motor Control Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

To evaluate reorganization of body part representations in human motor cortex after amputations, we studied motor evoked potentials delivered by a Cadwell MES-10 magnetic stimulator through a coil targeted over the motor cortex after amputations. We studied motor evoked potentials (MEPs) to conventional electrical stimulation and magnetic stimulation. The advantages of PMC stimulation were lack of precision in defining exact site of the nerve activation, and prolonged averaging time due to the slow capacitor discharge of the magnetic stimulator.
were recorded over 450 msec from 16 scalp electrodes. Moreover, 6 of the latter 10 P300s were present in some

Computerized four point linear interpolation was done to

S.B. Seminara*, and S. Khos'nbin. EEG Lab., Brigham

INDICATIONS OF A TEMPORAL LOBE ORIGIN OF THE P300 WAVE.

bursts were recorded in 8 normal subjects. Stimuli were

presented at 60dB above hearing threshold to right and

tro-consulsive therapy (ECT). To test this hypothesis,

ES obtained after each electrically induced seizure as

relation was found between the average degree of electri­

and the degree of improvement on the HDRS after six treat­

measured on diabetics (N=78) and normals (N=52). Since it has been

high pitch tones (2000Hz) and ignore 85% of low pitch tones

retinopathy. The subjects were required to pay attention to 15% of

312.22

INFORMATION PROCESSING AND DIABETES MELLITUS.

J. Everett¹, M.A. Kabbes², C. Harnois², (SPON: A.P. Caspule) School of Psychology¹, and Department of Ophthalmology², Laval University, Quebec City, Canada.

In order to assess the effect of Diabetes Mellitus on information processing capacities, event related potentials (N100 and P300) were measured on diabetics (N=78) and normals (N=52). Since it has been well documented that similarities between the retina and the brain exist, diabetics were divided into two groups: with (N=34) and without retinopathy (N=44). To assess the possibility that the possible brain impairment found in diabetics may occur in parallel with retinopathy. The subjects were required to pay attention to 15% of high pitch tones (2000Hz) and ignore 85% of low pitch tones (1000Hz) emitted randomly. The electrodes were placed at C3, C4, Pz (reference) and Fz (ground). While no differences were found for N100 and P300 amplitudes of improvement on the P3, the N100 and P300 amplitudes of improvement on the P3 were significantly larger in N1P2 and N2P3 amplitudes comparatively to controls. No differences were found between diabetics with or without retinopathy. These results seem to indicate selective attention deficits in diabetics regardless of their retinal status.

Exogenous corticosteroids are essential for moderating many detrimental side effects associated with brain tumor treatment. However, they may also have a serious negative treatment effect, such as the sparing of tumor tissue (membrane stabilization) and a decreased immune response, which could otherwise result in more beneficial treatment. In the present study we evaluated the effect of different corticosteroids on the experimental hyperthermia/mild hypothermia and/or radiation treatment being assessed. Based on clinical signs and MR/CT imaging it was observed that therapeutically relevant doses of dexamethasone and methylprednisolone caused a marked increase in the resistance to the effects of radiation and/or shortwave radiation. However, corticosteroid treatment also resulted in a decreased immune response, which could otherwise be beneficial for the treatment of brain tumors. Therefore, the resistance to frequency depression of reflexes in the present study, which was not detected in the DTR, suggests that presynaptic inhibition was significantly reduced in the contused SCI & non-SCI subjects. Both the SCI and contused animals demonstrated neurophysiological change associated with bladder distention in SCI subjects, which was not detected by the DTR. The results indicate that DTR "hyperreflexia" is not a feature of spasticity due to SCI.

REFLEX CHARACTERISTICS OF SPINAL CORD INJURED (SCI) HUMANS. P.W. Rance, Dalhousie University, Halifax, Nova Scotia Canada B3H 4K4.

With a solenoid-driven hammer which delivers variable intensity of force from 1-10, the deep tendon reflex (DTR) force response curves were recorded for SCI & non-SCI subjects. Both groups showed graded responses such that the greater the intensity of impact force the larger the DTR amplitude. In summary, the DTR "hyperreflexia" is not a feature of spasticity due to SCI.

NEW METHODS TO ESTIMATE THE NUMBER OF MOTOR UNITS IN A HUMAN HAND MUSCLE. J.F. Yang, R.B. Stein and J. Juncadella. Division of Neuroscience, University of Alberta, Edmonton, Canada T6G 2G2.

The number of motor units in the thenar muscle was estimated using 3 independent methods. The maximum surface electromyogram (SMEG) and switch response to stimulation of the median nerve was assumed to represent recruitment of all motor units in that muscle. The number of units was estimated by dividing the maximum observed muscle EMG response by the estimated single unit contribution. Single unit contributions to the SMEG and force were obtained separately by spike triggering averaging (STA), intramuscular motor unit detection (Motin) and graded whole nerve stimulation. Approximately 20 units were used to estimate the average single unit size for each method, in each subject. The estimated number of units in 8 normal subjects ranged from 100 to 200 using both the Motin and STA methods, in reasonable agreement with histological reports. The estimates based on whole nerve stimulation were more variable, ranging from 100 to 400. Results from 8 patients with cervical spinal cord injury suggested that methods which relied upon motor units, while others had severe motor unit loss with corresponding enlargement of the surviving units. Both the Motin and STA methods provided reasonably estimate in normals and were sufficiently sensitive to detect alterations in patients.

MAGNETIC RESONANCE IMAGING (MRI) OF FETAL CAT NEURAL TISSUE TRANSPLANTS IN THE ADULT CAT SPINAL CORD. E.D. With, T.H. Hanser*, D.P. Theile*, C.A. Brown*, D.K. Anderson and P.J. Reier. Dept. of Neuroscience, Neurosurgery and Radiology, Univ. of Florida College of Medicine, Gainesville, FL 32610 and VA Medical Center, Cincinnati, Ohio.

MRI was evaluated for its potential to detect the survival of fetal neural grafts in the adult cat spinal cord (SC). Three adult female cats received a hemisection at either the T11 or L2 level, followed immediately by implantation of either E17 fetal SC or cerebral cortex. Histological, histochemical and autoradiographic techniques were used to assess graft survival. In a fourth cat a static load compression (i.e., conus) lesion was made at the L2 level. Seven weeks post-implantation, the lesion was resected and fetal brain tissue with isofluorane (1% in 100% O2). MRI was performed on a 2.0T system with a quadrature surface coil and the cat in the stationary position. Multiphase spin-echo images (TR/TE = 1000/30) were obtained in both the transverse and sagittal planes. The transplant site was imaged with T2-weighted images, the host SC and grafts could be readily distinguished. The graft in the contused cat and two of the histected animals received a perfusion (0.2 mg/kg) 1 day prior to transplantation and daily thereafter. Six months post-implantation, the brains were sectioned into 10 mm slices and then stained with a 0.5% solution of 4',6-diamidino-2-phenylindole (DAPI) and Xyladine (3.2 mg/kg) and Xyline (0.22 mg/kg) and maintained during imaging with Isoflurane (1% in 100% O2). MRI was performed on a 2.0T system with a quadrature surface coil and the cat in the stationary position. Multiphase spin-echo images (TR/TE = 1000/30) were obtained in both the transverse and sagittal planes. The transplant site was imaged with T2-weighted images, the host SC and grafts could be readily distinguished. The graft in the contused cat appeared as a region of hypointensity within the SC, whereas the grafts in the histected cat SCs appeared as hyperintense areas. Two of the cats were perfused immediately following MRI imaging, one with 4% paraformaldehyde and one with 4% paraformaldehyde and 0.02% picric acid. The histological studies confirmed the presence of viable graft tissue, with the rostral and caudal border of the transplant corresponding to the MR images obtained. Therefore, MRI can be used to non-invasively demonstrate the presence of viable transplanted neural tissue in both the histected and contused spinal cord. MRI should prove to be a useful tool to study graft survival and morphology, and to assess graft-mediated functional repair of the injured spinal cord.

We are analyzing the growth and differentiation of intraspinal neural tissue transplanted into the spinal cord (SC) of the adult cat. In 4 cats the SC was compressed at the L2 level, 1 week after trauma. The impacted SC was removed and either T3 or T7 fetal cat neural tissue, or brainstem or SC was implanted into this cavity. In 5 cats, the SC was hemicorpectomized and similarly transplanted. Both of the cats received oral cyclosporine (10 mg/kg) one day prior to transplantation and daily thereafter. At either 6 weeks, 12 weeks or 5 months, the cats were perfused, fixed and the SC analyzed by light and electron microscopy. Large grafts filled the lesion cavities and showed extensive vascularization without signs of necrosis or rejection. Cells in the neocortical grafts appeared immature and the surrounding neuropil was unmyelinated. Regions of close approximation were seen with no scar formation. The T7 SC grafts were large with histological features of the normal SC. These grafts were more mature and less integrated with the host than the neocortical grafts. These findings indicate that intraspinal transplantation is feasible in acute and chronic lesions of the adult cat SC and that this model can be used to study functional repair of the injured SC.

487.5 ANALYSIS OF RATS RECEIVING FETAL SPINAL CORD TRANSPLANTS SUBSEQUENT TO MID-THORACIC CONDUCTIVE SPINAL INJURY. D.L. Winalski and P.J. Reier (UPN: Wm. Friedman). Deps. of Neuroscience and Neurosurgery, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ 08903.

We have previously reported evidence that fetal CNS grafts could repair sites of tissue damage in longstanding contusion injuries of the adult rat spinal cord. Only partial reconstruction was achieved, however, as cavitatory cavities and dense astrocytic scarring were present at the level of injury. In the present study, we extended our observations to examine fetal spinal cord tissue in the epifocal area of the contused spinal cord. Using a modified tailweight-drop apparatus, contusion injuries were made at the T8-9 vertebral level. After post-injury delays of either 14 days (N=22) or 1 week (N=7), dissociated E14 spinal cord cells (20 ul of cell pellet per fetal spinal cord) were injected into the lesions of 0.5 ul each were made, yielding a total injection volume of 20 ul per recipient. After post-injury survival periods of 1-6 weeks, animals were sacrificed, spinal cord embeddings were placed in plastic, and 2 um sections were examined. At 1 week post-injection, in either the 14 days or 1 week post-injury recipients, relative undifferentiated fetal tissue survived at the injection site and exhibited some degree of degenerative differentiation. Large patches of donor tissue were also often observed at levels immediately subjacent to the lesion. However, no evidence of glial scar tissue was present in these donor tissues.

487.6 EFFECTS OF GABA ON NEURONS IN INTRAOCULAR SPINAL CORD TRANSPLANTS. J. G. Broton, R.P. Vesseryk, and A. Seiger. Dept. of Neurological Surgery, University of Miami, Miami, FL 33136.

Gamma-amino-butyric acid (GABA) is present in the normal rat spinal cord. We have previously reported that grafts of fetal spinal cord tissue transplanted into visual cortex of neonatal rats had a variable number of GABA-IR neurons. In this current study we have characterized the effects of GABA administration on spontaneously-active spinal cord co-grafted neurons within the visual cortex.

Intraocular spinal cord grafts were transplanted into adult rats. GABA was applied topically to the wall of the graft, and the effects of GABA application on neuronal activity observed. GABA increased the firing rate of all 12 neurons. The mean increase in firing rate was 5.2 Hz, with the maximum change observed in the temporal lobe. GABA receptor agonist bicuculline methiodide (1-10mM) increased the firing rate of all 12 neurons tested.


Intraocular grafting of neuronal tissue has been used as a model to study differentiation and growth of spinal cord areas, including the spinal cord. In this study, fetal spinal cord grafts were transplanted and maintained in ocular chambers for several months. Host animals were anesthetized and perfused with formaldehyde. Grafts were removed, fixed and processed at 30um, and processed for immunohistochemistry with antisera against N-acetylasparglutamate (NAG), glutamate, and glutamate dehydrogenase (GDH). NaAG and glutamate-immunoreactive (IR) neurons were found in most grafts. Some large neurons resembling motor neurons were observed. GABA-IR and GABA-IR astrocytes were also observed. The results of this study indicate that neurons and astrocytes that contain markers for cells involved in excitatory amino acid metabolism continue to develop and differentiate within intragrafts. The intragraft paradigm may be a useful model to study growth in excitatory amino acid neuronal systems with the support of Miami Project to Cure Paralysis.

The classic monamine theory of depression states that depression may be caused by a central deficiency of monoamines and serotonin and that antidepressant and electroconvulsive shock (ECS) therapy work by correcting this deficiency. One of the most reliable and widely accepted animal models of depression is the learned helpless (LH) model. The LH model has been shown to demonstrate good predictive validity for the treatment of human depression as it is reversed by antidepressant and ECS therapy. ECT research has demonstrated that the antidepressant desipramine prevents the development of LH in rats when injected directly into the frontal neocortex. Adrenergic monoaminergic neurones have been shown to release dopamine and noradrenaline in these regions including norepinephrine, and the pineal gland has a very high concentration of brain serotonin. We transplanted either rat adrenal medullary, rat pineal gland tissue, a combination of adrenal and pineal grafts, in contrast, depression was not prevented by control striated muscle tissue grafts to the frontal neocortex. Morphological studies revealed that greater monoaminergic tissues survived well and continued to produce high levels of monoamines. These results suggest that neural transplants can provide a long-term source of monoamines for reducing depression.


Previous work involving intraparenchymal xenografts of bovine chromaffin cells into the CNS has been characterized by low survival rates that indicated cell loss, in contrast to whole tissue implants, allow for greater host-graft integration. In an attempt to enhance survival and integration of the transplanted cells, we have treated various tissue samples with several agents known for their ability to promote graft survival and enhancement of host-graft integration. These agents included nerve growth factor (NGF), ganglioside GM1, and the immunosuppressive agent cyclosporin A, administered alone or in combination. Isolated bovine chromaffin cells in primary culture were stereotypically observed to grow into the rat brain in the presence of these agents. We proved daily injections of cyclosporin A for 3, 6, or 12 weeks. Perfusion for immunocytochemical or EM analysis was done either immediately following termination of the experiment or delayed until the end of the 12 week period.

Marked increased survivability was observed in all animals receiving cyclosporin A treatment, compared to untreated animals. This marked improvement in cell survival was attributed to the administration of cyclosporin A, which is known to enhance graft survival. In some cases, this was evidenced by the presence of nerve terminals within the graft tissue, indicating a higher degree of host-graft integration.


Work in our laboratory has shown that pain sensitivity induced by transplanted adrenergic medullary chromaffin cells into the spinal cord subarachnoid space. The effect of low doses of nicotine on pain sensitivity in these animals. The analgesia most likely results from the released opioid peptides from the transplanted cells, since it can be blocked by the opiate antagonist naloxone. Furthermore, the duration of analgesia following nicotine can be prolonged by kelatorphan, an enkephalinase inhibitor. Since a significant therapeutic problem with opiates is the development of tolerance, the purpose of this study was to determine the extent of tolerance development to the transplants. Dose-response relationships to several doses of nicotine (0.05 - 0.2 mg/kg, s.c.) and morphine (1 - 10 mg/kg, s.c.) were determined using analgesimetric tests prior to and following the transplantation of adrenergic medullary or control tissue. In addition, some animals were implanted subcutaneously with nicotine pellets containing several doses of nicotine (0.02 - 100 mg released over a three week period) and tested for dose-responsiveness to acute nicotine or morphine injection. Results indicated that adrenal medullary implants shifted the morphine dose-response curve to the left, suggesting that, not only is there no cross tolerance to morphine, but the dose-responsiveness to morphine is also potentiated by the adrenal medullary implants. Following implantation of nicotine pellets, dose-response curves to acute nicotine injections were shifted to the right by high pellet doses, while responses to morphine injections were unaltered. These results suggest that transplanted cell surface nicotinic receptors are less responsive when constantly stimulated by high doses of nicotine, but there is little tolerance at the host opiate receptor. (Supported by NIH grant NS25054).

LIMBIC SYSTEM II

488.1 A TIMM STAIN FOR ZINC WITHOUT SULFIDE PERFUSION M.D. Haigh*, C.J. Frederickson, G.A. Howell, (SPON: B.P. Johns) Lab for Neurobiology, Univ. of Texas/Dallas, Richardson, TX. 75083.

A previously described post-mortem Timm method (Chafetz, 1986, Brain Res. Bull.) has been modified to produce visible zinc staining in the brain that are consistent with traditional Neo-Timm’s histochemistry. Specific modifications include (1) increasing the concentration of Na2S from 0.37% to 2%, (2) changing from a phosphate buffer to TRIS, (3) soaking slides in Na2S for 30 sec rather than 1 min, (4) dipping slides in 5% potassium permanganate rather than 2% potassium dichromate, and removing any metallic contamination.

The zinc staining patterns that are obtained with this modification are directly comparable to those obtained by perfusion with sodium sulfide in that the hippocampal subfields can be differentiated (including the inner and outer molecular zones of the fascia dentata) and cell bodies are unstained. The methodological improvements result in a post-mortem suicide stain that is a useful addition to the study of zinc histochemistry in the brain, with potential applications in the study of a variety of human pathological conditions.


Timm-Danscher histochemistry shows that many limbic and cerebrocortical regions are rich in metal-containing axonal boutons. The fluorescent marker for zinc, TQS (Molecular Probes), indicates that zinc is the metal in most of these regions. In this study, we used ZnTQS fluorescence to estimate the relative abundance of zinc-containing boutons in 13 rat brain regions. Probenecid (500 mg/kg, s.c.) was co-administered with TQS and fluorescence (500 nm; 55 um sampling spot) was measured using microspectrophotometry. Compared to the ZnTQS fluorescence from the zinc-rich hilus of the dentate gyrus, structures such as the lateral amygdala (fluorescence intensity 4% of hilus) showed relatively high fluorescence. Neocortical laminae II-III (i = 21%) and V (i = 13%) showed only a little fluorescence, whereas structures that are essentially unstained by Timm-Danscher methods gave readings near zero (corpus callosum i = 1.9%, neocerebellar cortex = 0.9%). The data emphasize that zinc-containing fiber systems are abundant throughout the forebrain.

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The mamillary tract (MTG) is a major descending pathway for conveying information from the mamillary body (MB) and other limbic system structures to the brain stem. Although the broad outlines of descending projections from the MB (Guillery, 57; Crue, 77) are known, little is known about the fine structural organization of MTG endings in the midbrain tegmentum (Takahashi et al., 85). In the present study, the fine structural synaptic organization of MTG fibers in the midbrain tegmentum was analyzed following injections of WGA-HRP into the MB. The study was limited to the medioventral portion of the anterior mamillary nucleus product of 4-aminobenzenesulfonyl fluoride (TMB), diaminobenzidine (DAB), TMB stabilized with DAB or TMB stabilized with ammonium molybdate and processed for light and electron microscopy. After injection of tracer into the lateral and meddial mammary nuclei, dense anterograde and retrograde labeling were observed in the dorsal and ventral tegmental nuclei, respectively, and dense topographically organized anterograde labeling was observed extending from the medial portion of the nucleus resiliarius tegmenti pontis into the instal medial pontine nuclei. At the electron microscopic level, labeled axon terminals were observed in the dorsal and ventral tegmental nucleus and the nucleus resiliarius tegmenti pontis and pontine nuclei. The labeled terminals were small (diameter <2 µm), contained mainly round synaptic vesicles and formed mainly asymmetric synaptic junctions with small diameter dendritic profiles and occasionally with neuronal somata in the dorsal and ventral tegmental nuclei. The presence of labeled terminals on labeled preterminal elements in the midbrain indicates that cells which project to the MB receive direct reciprocal inputs from the MB. In the present results indicate that mamillary terminations in the midbrain are located primarily on dorsal dendrites and are most likely excitatory in nature. Supported by MRC of Canada.


Nucleus accumbens lesions impair the organization of behavioral sequences such as when in rats (Kelley & Substance, 1985, Behav. Neurosci. 99: 357). The present study characterizes, particularly at the ultrastructural level. The present study employed injections of PHA-L to label hippocampal afferents to the nucleus accumbens. Male Sprague-Dawley rats were anesthetized with pentobarbital and stereotaxically injected with a 2.5% solution of rabbit anti-PHAL antibody for 48 hours and then processed for immunoperoxidase labeling. The NA lesioned animals, which were able to pick up and store the nuts when the NA lesion significantly affected GAD levels in the medial dium of the MN. These results suggest that the principal GABAergic input to the MN arises in the VTN and that the ascending DTN fibers pass near the VTN en route to the LM. Additionally, GABAergic afferents from the VTN appear to modulate the VP input to the MN. The present results substantiate the hypothesis that the NA lesion model provides a reliable tool for studying the organization of behavioral sequences in primates.


Although a number of neuroanatomical studies suggest projections from the NAS to the VP, attempts to antidromically label single units by VP stimulation have indicated only a small percentage (10%) of VP neurons as NAS neurons as VP projection cells. Additionally, recent neuroanatomical reports have suggested that the VP typically projects back to the NAS. As part of ongoing electrophysiological studies of the NAS in anesthetized rats, we have observed that the NAS does project to the VP and that the VP reciprocally projects back to the NAS. Analyzing the afferent and monosynaptic orthodromic effects of VP stimulation it is also apparent that these projections show a significant mediod-lateral topography with the VP-NAS projection cells are found in the lateral NAS while NAS-NAS projection cells are found predominantly in the medial NAS. Further evidence of this reciprocal circuitry is also found in subsequent experiments showing both orthodromic and antidromic responses in cells recorded within the VP following stimulation of the NAS. The concurrent analysis of VP and fornix input to the NAS suggests that these inputs interact, possibly in a monosynaptic convergence upon the same NAS neurons. Further analysis of these interactions should help elucidate NAS-NAS integration processes. (Supported by DA03565 and KO2/DA00131 to S.H.)
488.9

We injected Phaseolus vulgaris-leucoagglutinin (PHA-L) in the nuclei of the thalamus of the rat to study the projections of this nucleus to the hippocampus and parahippocampal areas. At the LM-level, we combined the tracing with immunohistochemistry of GABA, vasoactive intestinal peptide (VIP), and neuropeptide-V (NPY). There is dense homogeneous labeling in the stratum lacunosum-moleculare of the entire hippocampal field CA1, the molecular layer of the dorsal and ventral subiculum, layer I of the para- and presubiculum, layers I, III and IV of the dorsolateral entorhinal cortex (DLEA), layers III-V of the ventrolateral entorhinal cortex, layer I of the perirhinal cortex, and layers III-IV of the medial entorhinal cortex (MEA). The labeling is clustered in layer I of the MEA and the caudal part of the DLEA. GABA-immunoreactive cell bodies are in the areas of the termination are frequently apposed by PHA-L-labeled fibers, whereas cell bodies immunoreactive for VIP or NPY are not. At the EM level, the axon terminals of the thalamohippocampal fibers form asymmetric synaptic contacts with dendritic spines and thin shafts of spinous dendrites and contain spherical synaptic vesicles.

488.11
FUNCTIONAL PROJECTIONS FROM THE RAT HIPPOCAMPAL FORMATION TO THE MEDIAL FRONTAL CORTEX: AN IN VIVO INTRACELLULAR STUDY. T.D. White, A.M. Tang, and D.M. Finch. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

Recordings of long latency (often > 20 ms) inhibitory (47%) and excitatory (4%) post-synaptic potentials were obtained from principal neurons (N=69) in medial frontal cortex (MFC) in response to single shots of the hippocampal formation (HF). Yet, principal neurons (N=61) in the HF were frequently (59%) antidromically activated by stimulation of the MFC. These results indicate that a significant oligosynaptic projection from the HF provides feedforward inhibition to the MFC. In addition, five candidate inhibitory neurons (CIN) were identified in MFC which responded to electrical stimulation of the HF. One class of CIN (N=4) exhibited short duration bursts of action potentials followed by inhibition. A second class of CIN (N=1) responded with a long duration burst of action potentials without subsequent inhibition. The excitatory bursts of the first class of CIN corresponded to the CI-dependent inhibitory post-synaptic potentials of principal cells. The excitatory burst of the second class of CIN corresponded to the non CI-dependent inhibitory postsynaptic potentials of principal neurons and to the duration of inhibition in the first class of CIN. A model of MFC circuitry is presented. Supported by NIH Grant NS 16721.

488.12
FUNCTIONAL PROJECTIONS FROM THE RAT MEDIAL FRONTAL CORTEX TO THE ENTORHINAL CORTEX AND SUBICULAR COMPLEX. AN IN VIVO INTRACELLULAR STUDY. D.M. Finch, A. M. Tang, and D. M. White. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

A number of recent anatomical studies have shown extensive projections from medial cortex to the entorhinal cortex and subicular complex. Since there are virtually no cellular physiological studies of these pathways, we studied them using in vivo neurophysiological recording techniques. Sprague-Dawley albino rats (N=110) were anesthetized with chloral hydrate (400 mg/kg, i.p., supplemented by i.m. injections as necessary). Single-unit stimulation and recording electrodes were lowered stereotactically to their targets. A high proportion of neurons within the medial frontal cortex were antidromically activated at short latency (<1 msec) by electrical stimulation of the entorhinal cortex or subicular complex, indicating fast direct projections from the medial frontal cortex. The predominant synaptic response in entorhinal or subicular complex neurons after electrical stimulation of the medial frontal cortex was inhibition, as shown by the presence of CI-dependent EPSPs. A small percentage of entorhinal cortex and subicular complex principal cells showed clear EPSPs. Candidate inhibitory cells were also encountered. The results indicate the presence of a physiologically significant cortico-cortical association pathway between these two distant regions. Supported by NIH Grant NS 16721.

488.13

We have studied monosynaptic connections from the amygdala (A) to the entire neocortex of adult cats. Seventy-six animals received injections of WGA-HRP in A to trace a microaperture. Our results indicate that axons from magnocellular basal amygdaloid nucleus (ABMc) innervate a vast extent of the neocortex, encompassing premotor and motor areas, insular and perirhinal cortices, somatosensory areas SII and SIV, as well as ventral sectors of SI, auditory area AI, all sectors of the suprasylvian fringe, and visual areas Ps and Pmt. The major limbic areas and retrosplenial cortex were densely innervated, while area singularis (24) was almost free of amygdaloid connections. ABMc axons selectively reached deep part of layer I in both areas. They entered premotor areas II and III of motor cortex, premotor, and perirhinal cortices. Lateral amygdaloid nucleus projects to a much reduced field, including caudal insular cortex, perirhinal areas, ventral sectors of temporal association auditory fields as well as infralimbic and ventral prefrontal areas. The results provide an anatomical basis for selective monosynaptic input from the limbic system over a wide array of neocortical regions involved in sensory, association or motor processing. CAST/7 Grant 1988-0558 and FIS99-1623 (F.C.)

488.14

In an attempt to investigate limbic influence on the cerebral cortex, we have studied monosynaptic connections from the amygdala (A) to the entire neocortex of adult cats. Seventy-six animals received injections of HRP in discrete sectors covering the entire neocortical mantle. Twelve further animals received small deposits of WGA-HRP in A to trace a microaperture. Our results indicate that axons from magnocellular basal amygdaloid nucleus (ABMc) innervate a vast extent of the neocortex, encompassing premotor and motor areas, insular and perirhinal cortices, somatosensory areas SII and SIV, as well as ventral sectors of SI, auditory area AI, all sectors of the suprasylvian fringe, and visual areas Ps and Pmt. The major limbic areas and retrosplenial cortex were densely innervated, while area singularis (24) was almost free of amygdaloid connections. ABMc axons selectively reached deep part of layer I in both areas. They entered premotor areas II and III of motor cortex, premotor, and perirhinal cortices. Lateral amygdaloid nucleus projects to a much reduced field, including caudal insular cortex, perirhinal areas, ventral sectors of temporal association auditory fields as well as infralimbic and ventral prefrontal areas. The results indicate that orbital limbic, followed by medial cortices, have widespread connections with several amygdaloid nuclei, whereas the most differentiated prefrontal cortices have few and topographically restricted amygdaloid connections.

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RESPONSES OF AMPHIALOID CENTRAL NUCLEUS (ACE) NEURONS TO STIMULATION OF THE PARABRACHIAL NUCLEUS (PBN) IN RABBITS
J.P. Pascoe and R.S. Kapp. Department of Psychology, The University of Vermont, Burlington, VT 05405

The ACE is one essential component of a forebrain system that contributes to cardiovascular activity during emotionally arousing stimuli. It has reciprocal connections with the ACE and may be an important relay in both ascending and descending pathways between the ACE and lower brainstem cardiorespiratory nuclei. We are examining activity in ACE neurons during sensory stimulation and following single pulse stimulation of the PBN using our standard methods.

Activity in 71/127 ACE neurons was infrequent (4.0 ± 1.5 Hz) and was not increased to sensory stimuli. Of these, 19 were activated antidromically at latencies of 5 ± 4 ms (25 ± 14 ms), and 24 were activated synaptically at latencies of 8 ± 6 ms (26 ± 18 ms). Activity in 15 other slowly discharging neurons (1 Hz) was increased to sensory stimuli (143 ms), and 6 of these were activated synaptically at latencies of 8 ± 60 ms (264 ± 17 ms). Activity in 32 rapidly discharging neurons (19.3 Hz) was increased (86 ms, n=15), decreased (86 ms, n=10), or unchanged (n=7) to sensory stimuli, and PBN stimulation increased (n=19) or decreased (n=3) activity in 22 of these at latencies of 5±40 ms (17 ± 8 ± ms). Thus, activity in ACE afferents to the PBN is in similar to that of other ACE-brainstem efferents described previously (Pascoe & Kapp, 1987), and stimulation of the PBN often activates activity in ACE neurons. Supported by the American Heart Assn. and the ANA Vermont Affiliate, Inc.

METALLOTHIONEIN INDUCTION IN RAT HIPPOCAMPAL NEURONS IN PRIMARY CULTURE. P. Thakran*, M.P. Leuschner, M. Ebadi*. Dept. of Pediatrics and Pharmacology, Univ. of Neb. Med. Ctr., Omaha, NE.

Primary cultures of neurons are important tools for the study of neurotoxic processes. Our interest is in the role of trace elements in epileptogenesis and the influence of metal binding proteins in temporal lobe epilepsy. The induction of metallothionein occurs in a protein-rich protein believed to regulate the flow of essential trace elements through cellular compartments, has been studied in cell lines and tissues other than the brain. We have found that cultivating hippocampal neurons in the cysteine-free Iseove's modification of Dulbecco's MEM (IMDM) supplemented with 25 mM t-K, 300 µg/ml transferrin, 10 µg/ml BSA, 10^-12 M estradiol, 100 µg/ml gentamicin and 3 µg/ml insulin. Two cultures were utilized: Poly-L-Lysine and Collagen (derivatized to plastic culture dishes by a cross-linking reagent Carbodiimide). Poly-L-Lysine proved superior to Collagen as a substrate for neurons explanted at a comparatively later stage of development (60 days post-natal) showed earlier neurite outgrowth within 3-5 days compared to those from neonatal rats plated on collagen. Neurons were identified by histochemical staining for cholinesterase. All cultures survived for 3 weeks and induced metallothionein synthesis at zinc (at concentrations of 10^-10 to 10^-7 M) as judged by 35S-cysteine incorporation.

Maximum induction occurred after 48 hrs. incubation with zinc.


A neuronal-specific mRNA, expressed at high levels in CA3 pyramidal neurons of the mouse hippocampus (MuBl8, Brains and Wilson, Mod. Brain Res. Rev. 1, 1-56, 1986), encodes a novel cytoplasmic synaptic protein (SNAP-25) that potentially contains a zinc binding domain (Oyler et al., submitted). We examined the expression of SNAP-25 protein and mRNA in the rat hippocampal formation, using immunocytochemistry and in situ hybridization, following hippocampal injection of kainic acid or colchicine and following electrolytic lesions of the entorhinal cortex. Kainic acid lesions, which destroy pyramidal CA3 and hilar neurons, did not alter the pattern of SNAP-25 mRNA in CA3 but did result in decreased immunoreactivity in the inner 1/3 of the molecular layer of the dentate gyrus. Colchicine, which selectively removes the granule cells of the dentate gyrus, reduced SNAP-25 mRNA in CA3. Following electrolytic lesions of the entorhinal cortex, SNAP-25 immunoreactivity in the inner molecular layer was intensified and expanded to occupy the inner 1/2 of this region, consistent with a sprouting of the commissural/associational system which originates in the hilar neurons. These results were similar to those observed using Timm's stain for zinc and demonstrate that SNAP-25 is located within the presynaptic terminals of the mossy fibers and of the mossy cell projections to the molecular layer of the dentate gyrus. SNAP-25 is a novel presynaptic marker of select hippocampal pathways that may be useful in studies of neurological disorders such as Alzheimer's disease. Supported by NIA LEAD Award and by NIH Grant NS2303A.


In the brain basic FGF has been shown to have neurotropic action both in vivo and in vitro. We have previously shown in situ hybridization that basic FGF mRNA is distributed in neurons localized primarily in the CA2 region of the hippocampus. Since this region densely concentrates steroids such as glucocorticoids, the purpose of this experiment was to investigate the effect of ADX on the distribution of basic FGF mRNA in the hippocampus. Male adult rats were adrenalectomized, sham adrenalectomized or given corticosterone (B) replacement plus adrenalectomized. Distribution of basic FGF mRNA was examined using in situ hybridization one week after surgery. Animals were perfused with paraformaldehyde using the pH shift method. 25-µm sections were mounted on polylysine-coated slides. The rat basic FGF clone R0 basic FGF was subcloned into plasmid SK+ and anti-sense probe was transcribed using T7 RNA polymerase and 35S-UTP. The upstream sense strand was used as a control. After hybridization, sections were treated with ribonuclease A and washed in 0.1 SSC at 55°C. Slides were exposed to Kodak NTB-2 autoradiograph emulsion for 4 weeks. Results showed that basic FGF mRNA was distributed in the hippocampus of control, adrenalectomized and adrenalectomized-B-treated rats primarily in the CA2 region. This agrees with our previous results in 90-day-old male rats. Surprisingly, signal was also observed in the CA1 region. In summary, it appears that short-term ADX has no effect on hippocampal distribution of basic FGF mRNA. An effect of long-term ADX on distribution of basic FGF mRNA cannot be excluded.


Patients with global ischemia due to a circumscribed memory loss and selective hippocampal CA damage. In the rat (4-V0) model of stroke, memory loss and CA damage occur after 30 min. of ischemia. The CA2 region contains major intrahippocampal integration and CA neurons major efferent hippocampal information output. The aims of this study were to compare differential CA-C3 cell vulnerability after 30 min. of 4-V0 ischemia. Damage was evaluated in 6 µm sections, stained with H & E, 16 days after 4-V0. BM201P programs were used to compare CA1-C3 cell damage. 4-V0 ischemia produced highly significant CA1 (p < 0.001) and lesser but significant CA3 (p < 0.01) cell damage. Damage in CA1 was significantly (p < 0.01) greater than in CA3. Gradients of hippocampal cell vulnerability depended upon duration of 4-V0 ischemia, vascular, and cellular factors. Studies have been undertaken to determine the extent to which ischaemically compromised but viable CA1-CA3 neurons may be amenable to drug modification in stroke.
489.5

We investigated hippocampal commissural projections in the neonatal rat using retrogradely transported rhodamine labelled microspheres. (Retrograde tracing).

Sprague-Dawley rat pups (ages 3-5 days) were injected with 0.1-0.5 μl of retrograde tracer, axonal transport was allowed for 48 hours and then animals were killed. Tissue was fixed by perfusion and the brain was processed for fluorescent microscopy to determine site of injection and contralateral transport.

In 3 of 4 CA1 injections (each injection represented one brain), labeled fibers were identified in the homotopic contralateral CA1 field. In 3 of 4 CA3 injections, label was present only in CA3. All 5 injections into the right hippocampus produced labeling. This labeling was seen in 2 brains and contralateral homotopic labeling. Labeling injections into the ventricular system did not label either hippocampus. When the commissural pathway was cut prior to CA1 injection, no label appeared in the contralateral hippocampus.

These results suggest that hippocampal commissural projections exist in the neonate, but the CA1 commissural projection present in the adult was not demonstrated. Further, a CA1 to CA projection was noted. This pathway has not been consistently reported in the adult.

489.6
ANTEROGRADE TRACING WITH BIOCYTIN DEMONSTRATES A PRECISE CORRESPONDENCE BETWEEN THE DENTATE GYRUS INNER MOLECULAR LAYER AND TMT'S STAIN BRAIN STEM. M. Zürcher*, Birmingham, Birmingham, AL 35294.

The Timm's sulfide-silicate technique reveals the location of certain metals in brain tissue, for example, the 3 bands in the so-called dentate gyrus of the hippocampus. Although these bands are generally considered to represent the synaptic terminal fields of particular dentate afferents, this identification has not yet been tested. We used biocytin to anterogradely label hilar afferents to the dentate M.L in brains that were also stained by the Timm's method. Adult male Long-Evans and Wistar rats were anesthetized with nembutal and brain tissue was immediately dissected into the skull. Biocytin was pressure injected into the dorsal dentate hilus and perfused with Formalin 1-4 days postinjection and perfused using the Timm's fixation protocol. Postfixed brains were Vibratome sectioned at 40 microns and sections reacted with avidinylated peroxidase or alkaline phosphatase followed by their respective chromogens. Precise correspondence was always observed between the inner ML Timm's stain band and the anterogradely labeled hilar axons and terminals, confirming that this Timm's band is a reliable marker for the hilar afferent terminal zone in the inner ML. Supported by the Veterans Administration and NINDS grant A02020.

489.7
RECURRENT MOSSY FIBER COLLATERALS IN THE HUMAN HIPPOCAMPUS: A QUANTITATIVE STUDY. C. Thalman*, H. Lipp, D.P. Wolfer* and U. Zöllner*. Institute of Anatomy and Institute of Forensic Medicine, University of Zürich, Switzerland.

Hippocampal mossy fibers emi collaterals (MFC) which dynamically innervate the granule cell layer (CGL) and a supragranular zone (SOG) of the fascia dentata. Lesions in rats have been reported to cause adult sprouting. In guinea pigs, the MFC have a defined growth spmns around puberty and in the midle period (Wolfer and Lipp, Soc. Neurol. Aust. 1981). In aged human brains, exuberant growth has been reported (Collins and M. Coma, Newsl. Neurol. 1982). An exuberant growth of mossy fiber axons has been also associated with epilepsy (Saalma et al., Science 197: 1147, 1982). For any comparison, however, it is necessary to know the area and distribution of MFC at different ages. We measured the results of a systematic quantification.

Nineteen hippocampi (age range 14-90 years, no documented neurological problems) were sectioned in a parasagittal plane along the unco-septal axis. Every 10th cryostat section of the hippocampal formation was examined using a fluorescence microscope to determine site of injection.

The Timm's sulfide-silicate technique reveals the location of certain metals in brain tissue, for example, the 3 bands in the so-called dentate gyrus of the hippocampus. Although these bands are generally considered to represent the synaptic terminal fields of particular dentate afferents, this identification has not yet been tested. We used biocytin to anterogradely label hilar afferents to the dentate M.L in brains that were also stained by the Timm's method. Adult male Long-Evans and Wistar rats were anesthetized with nembutal and brain tissue was immediately dissected into the skull. Biocytin was pressure injected into the dorsal dentate hilus and perfused with Formalin 1-4 days postinjection and perfused using the Timm's fixation protocol. Postfixed brains were Vibratome sectioned at 40 microns and sections reacted with avidinylated peroxidase or alkaline phosphatase followed by their respective chromogens. Precise correspondence was always observed between the inner ML Timm's stain band and the anterogradely labeled hilar axons and terminals, confirming that this Timm's band is a reliable marker for the hilar afferent terminal zone in the inner ML. Supported by the Veterans Administration and NINDS grant A02020.

489.8
ANTEROGRADE TRACING WITH BIOCYTIN DEMONSTRATES A PRECISE CORRESPONDENCE BETWEEN THE DENTATE GYRUS INNER MOLECULAR LAYER AND TMT'S STAIN BRAIN STEM. M. Zürcher*, Birmingham, Birmingham, AL 35294.

The Timm's sulfide-silicate technique reveals the location of certain metals in brain tissue, for example, the 3 bands in the so-called dentate gyrus of the hippocampus. Although these bands are generally considered to represent the synaptic terminal fields of particular dentate afferents, this identification has not yet been tested. We used biocytin to anterogradely label hilar afferents to the dentate M.L in brains that were also stained by the Timm's method. Adult male Long-Evans and Wistar rats were anesthetized with nembutal and brain tissue was immediately dissected into the skull. Biocytin was pressure injected into the dorsal dentate hilus and perfused with Formalin 1-4 days postinjection and perfused using the Timm's fixation protocol. Postfixed brains were Vibratome sectioned at 40 microns and sections reacted with avidinylated peroxidase or alkaline phosphatase followed by their respective chromogens. Precise correspondence was always observed between the inner ML Timm's stain band and the anterogradely labeled hilar axons and terminals, confirming that this Timm's band is a reliable marker for the hilar afferent terminal zone in the inner ML. Supported by the Veterans Administration and NINDS grant A02020.

489.9

Most research into the extrinsic projections of the hippocampus proper in the rat has focused on the contribution of pyramidal neurons in area CA3, and relatively little attention has been given to the potential contribution of non-pyramidal neurons to these projections. Recent studies in our laboratory indicated that the hippocampal projections to the retrosplenial cortex originate in partly in these so-called "interneurons". To further investigate the projections from these non-pyramidal neurons, retrograde tracing studies were conducted; injections of fast blue and fluoro-ruby were made into the regions to which the hippocampus projects (i.e. the retrosplenial cortex, entorhinal, and the pre- and parasubicural cortices) labeled non-pyramidal cells that were at the border of stratum radiatum and radiatum in both area CA3 and CA1. Each of these injections labeled neurons in the pyramidal cell layer of area CA3, that were relatively confined to a small region of the septo-temporal axis of the hippocampus. In contrast, the labeled non-pyramidal neurons were distributed with the septo-temporal axis of the hippocampus. Injection of the hippocampus into several subcortical areas, e.g. the nucleus accumbens, the lateral septal nucleus and the horizontal and ventral limit of the diagonal band of Broca, also retrogradely labeled non-pyramidal neurons in the hippocampus. Injection of the hippocampus labeled non-pyramidal neurons labeled by these injections were in stratum radiatum and oriens in area CA1 and the subiculum, and the labeled non-pyramidal neurons were confined to those regions that receive hippocampal afferents. These results demonstrate that the putatively inhibitory interneurons of the hippocampus formation project outside of the hippocampal formation and that different groups of putative interneurons project to different areas.

489.10
TRAJECTORY OF COMMISSURAL FIBERS FROM ENTORHINAL CORTEX IN THE CAT. Donald Siwek* and Bertram Payne (SPON: M. Feldman). Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The purpose of the present study was to determine the trajectory of axons in the hippocampal formation using a monoclonal antibody against serotonin (Sera-Lab, U.K.), in combination with the biotin-avidin peroxidase technique. Three types of labeled axons were observed: thick, non-vascular fibers, fibers with small (<1µm), fustom varicosities, and fibers with large (2-7µm) varicosities. The density of the fibers in the different cytoarchitectonic regions of the hippocampal formation was determined using a computerized image analysis system, and density profiles of every region were created. A highly characteristic pattern of the distribution of serotonergic axons was observed: in the hippocampus, the highest density was measured in the stratum lacunosum-moleculare and in the stratum lacunosum-moleculare. There was remarkably high density in the subiculum, with relatively large pre and parasubiculum and CA1 regions. In the dentate gyrus the density of fibers was relatively low, except for a thin layer of axons subjacent to the granule cell layers. We have also studied the synaptic connections the serotonergic axons made. The large varicosite axones made asymmetrical connections with neurons found in the hilus of the dentate gyrus and subiculum, while the axons with small varicosities formed synaptic connections in an irregular manner.

The present results strongly suggest that the serotonergic system has a highly specific and differential role in the modulation of nervous activity in the hippocampal formation. The observations also extend recent reports regarding the dual organization of the serotonergic fibre system in the cortex. (Mulligan and Tork, J. Comp. Neurol. 270: 86,1988; Mamounas and Moliver, Exp. Neurol. 102: 23, 1988).

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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
The monkey hippocampal formation receives inputs from areas 35, 36, TF and TH but not from area TE. W.A. Suzuki*, R. Insausti and D.O. Amaral. The Salk Institute, La Jolla, CA 92037 and Dept. of Anatomy, Univ. Navarra, Spain.

The hippocampal formation receives most of its cortical sensory information from polymodal neurons distributed in the auditory, somatosensory, and visual cortices and temporal cortices through projections that terminate in the entorhinal cortex (Insausti et al., J. Comp. Neurol. 264:356-393, 1987). It has recently been suggested that the unimodal, visual area TE has direct, reciprocal connections with field CA1 of the hippocampus (Yukie and Iwai, Neuroni. Letts. 88: 6-10, 1988; Brain Res. Ext. to which these areas project directly to the hippocampal formation will undoubtedly influence conceptualization of the role of the hippocampus in memory function. To study the connectivity of field CA1, we injected retrograde tracers into the CA1/subicular border of the hippocampal formation in 3 Macaca fascicularis monkeys. In the inferior temporal lobe, most of the labeled cells were found in the medial half of both the perirhinal (areas 35 and 36) and parahippocampal (areas TF and TH) cortices; smaller numbers of labeled cells were observed in the lateral half of these fields (fields). No labeled cells were observed in area TE. Conversely, while glutamatergic injections into the perirhinal and parahippocampal cortices resulted in retrogradely labeled neurons in several of these cortical regions, an injection placed into area TE did not lead to such labeling. Our anatomical data therefore indicate that the CA1 field, like the entorhinal cortex, receives primarily polymodal sensory input.

489.13

ORGANIZATION OF AMYGDALOID PROJECTIONS TO VISUAL AREAS OF THE OCCIPITAL AND TEMPORAL LOBES IN THE MONKEY, D. G. Amaral and F. Nahm*. The Salk Institute, La Jolla, CA. 92037.

In previous anterograde tracing studies (Amaral and Price, 1981) direct projections were demonstrated from the amygdaloid complex to much of the visually-related cortex. To determine the location and topographic organization of the cells of origin for these projections, injections of the fluorescent retrograde tracer Fast Blue and Diaminofluorescein Yellow were made into different regions of the occipital and temporal lobes in 8 Macaca fascicularis monkeys. Histological sections through the amygdaloid complex were surveyed for the presence of single or double labeled neurons and these positions were plotted using a computer-aided digitizing system. Injections located in cortical regions ranging from area 17 caudally to anterior temporal lobe resulted in labeled cells in the amygdala. The largest number of retrogradely labeled cells was observed in the basal nucleus mainly in its dorsal or magnocellular region. The rostrocaudal focus of labeled cells in the basal nucleus was observed at the rostrocaudal position of the injection site in the temporal or occipital lobes. When injections were placed rostrally and ventromedially in the inferotemporal cortex, labeled cells were also observed in the accessory basal nucleus. Few double labeled cells were observed in these experiments even when the two injections were separated by as little as 3 mm. These studies confirm that the basal nucleus projects widely to visual regions of the temporal and occipital cortices and that individual cortical regions appear to be innervated by separate populations of amygdaloid neurons.

489.14

MORPHOLOGICAL CHARACTERISTICS OF MEDIULLARY PROJECTION NEURONS IN THE RAT CENTRAL AMYGDALOID NUCLEUS. C.J. Shi*, L. Modarelli* and M.D. Caswell* (Spon. T.C. Ritchie), Dept of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The central amygdaloid nucleus (Ce) contain five cytoarchitectonic zones, each containing specific cell types. Previous studies have demonstrated, that the distribution pattern of neurons project to the dorsal medulla overlaps several of these cytoarchitectonic zones. This suggests the presence of multiple types of Ce neurons projecting to the medulla. To attempt to identify the morphologies of Ce projection neurons, we have used a combined retrograde HRP tracing technique with the somatotopic organization of Ce. In these studies, supported by NIH grants NS 23074 and NS 16721 and CNPq (Brazil).

489.15


Recent studies (e.g. Cochetto and Saper, 1987) have divided the rat insular cortex into a rostroventral dysgranular zone and caudodorsal granular zone corresponding to special and general visceral sensory representations respectively. Several studies have reported projections to the central amygdaloid nucleus (Ce) from the insular cortex (IC) but there has been little information on the specific termination patterns of projections arising from specific insular subregions. Accordingly, 25 adult male Sprague-Dawley rats received injections of WGA-HRP into the rostral part of the IC. These injections were made in a mediolateral extent of the IC as defined by the lateral geniculate projection (Hossmann and others). The results demonstrate that there are two basic types of brainstem projection neurons distributed in the medial part of Ce; a medium-sized pyramiform neuron with long, rarely branching dendrites with a low to moderate spine density; and a bipolar type with few, if any spines. These findings suggest that projections from the Ce to the dorsal medulla arise from similar cell types, irrespective of their cytoarchitectonic location. (Supported by NS 21319).

489.16

IN VIVO CHARACTERIZATION OF AMYGDALAR PHYSIOLOGICAL RESPONSES TO BASAL FOREBRAIN AND HIPPOCAMPAL COMPLEX STIMULATION IN RATS. L. L. Meli*, A. M. Tan* and D. M. Fitch (SPON: M. Nuwer), Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Amygdalar responses to electrical stimulation of the basal forebrain and hippocampal complex were recorded in vivo from chloral hydrate anesthetized adult male Sprague-Dawley rats. Recordings were made antidromically from the GABAergic rat nucleus basalis. The results demonstrate that basal forebrain nuclei contain neurons that project to the amygdala, as indicated by the presence of the ipsilateral pyramidal cell population in the lateral amygdala and ventral aspect of the amygdala and dorsal nucleus. These neurons respond to electrical stimulation of the amygdala with a latency slightly shorter than the IPSP latency of neighboring cells, suggesting that they were inhibitory cells. Supported by NIH Grants NS 23074 and NS 16721 and CNPq (Brazil).
Selective ligands for the sigma receptor have been
mediated via the sigma receptor.
high affinity sigma ligand haloperidol (n-3). A moderate
naloxone or the N-methyl-d-aspartate antagonist amino-
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n-5 slices) increased field potential amplitude an
yet to be elucidated. The aim of this study was to test
HIPPOCAMPUS BY MEANS OF ANTI-PARVALBUMIN IMMUNOSTAINING
C. Brain & Strickland, H. Breakey Dept. of Anatomy, J.A. Goethe
University, D-6600 Frankfurt/M., 70, FRC
The pattern of cellular processes seen in Golgi impreg-
nations is used for classification of neurons. Golgi impreg-
nations of adult human hippocampi were rare and success-
full. Therefore the dendritic arborization pattern was
previously correlated with the lipofuscin pigment pattern.
Within the hippocampus, parvalbumin cells are to specific anatomical
connections in aging and neurodegenerative diseases. Five postmortem
human brains were obtained from neuropathologically normal patients.
Whole-mounted sections of the hippocampus were processed for parvalbumin
immunostaining. A compact bundle of labeled fibers courses from the stratum oriens to the stratum radiatum, is a characteristic of normal aging.
Injections of WGA-HRP were made into the lateral
amygdala, and stained for Nissl substance to study cytoarchitectural features. Adjacent sections were processed for AChE histochemistry. The combination of these methods yielded an accurate
parcellation of the amygdala. Volumetric measurements of each nucleus of the amygdala were then obtained. Initial measurements of nuclear volumes in the normal human amygdala revealed: lateral n. 32%, basolateral n. 15%, cortical n. and cortical transition areas 10%, central n. 3%, medial n. 2%. Preliminary examination of postmortem brains from AD patients reveals loss of AChE staining and atrophy of the entire amygdala that is more pronounced in certain nuclei. These methods will facilitate determination of the distribution of immunohistochemical markers, pathological lesions, and atrophy in the human amygdala. Supported by NIH HD17253 and AG10607.
490.3

Extracellular recording in freely moving animals is a powerful technique for the study of function in the hippocampus and the discovery of place-coded neurones and indirectly to the cognitive map theory of hippocampal function. Difficulties with the present techniques limit their usefulness. To achieve stable recordings it is necessary to use insulated 25µm microwires with flat tips. While these electrodes overcome the stability problem they give relatively poor isolation of units in densely packed cell layers. The recording range of the electrode includes electrical potentials from numerous cells, many of which are equidistant from the tip. This places serious limitations on the ability to unravel the coding of environmental information within the hippocampus. Our previous solution to this problem involved the use of a paired set of microelectrodes, the "tetrode," (McNaughton, B.L., O’Keefe, J., Bicans, C.A., J. Neurosci. Meth. 8:391-397) in which the two tips are close enough to the same population of neurones. Unit potentials appear nearly simultaneously on both electrodes, with differing amplitudes, possibly due to the distance of the neurones from the two electrodes. Generally three to five units could be distinguished by their relative amplitudes. The tetrode is a direct extension of the stereotrode in which four wires simultaneously record the same unit. It improves the resolution and increases the number of simultaneously recorded units to as many as ten. We have found several cases in which the stereotrode separates units that could not be distinguished by a stereotrode. Finally, the tetrode opens up the possibility of localising the source of a unit potential in three dimensions, shifting the focus of the study of interactions between a group of anatomically identified neurones.

490.4

Existing microelectrode arrays suitable for current-source density analysis are too short for chronic recordings from deep structures in primates. Safety considerations further require that the electrode be blunt-tipped and flexible, as well as biocompatible. Coherence of large (4 µm²) contacts at 3.5 mm intervals, and 42 small (0.004µA) contacts separated by 80 µm in a row near the electrode tip for laminar and multicell recordings. The conducting lines are continuous with a 15 cm ribbon cable ending in an end connector. Fabrication begins with evaporating titanium and then gold onto a flexible polyimide wafer. The metal is etched away except for the electrode pads and conducting lines. An insulating polymer is then deposited and removed above the pads. Platinum is electroplated onto the gold, and chlorided if desired. A layer of silicone varnish layers the electrodes. Recordings are made on the electrode pads facing out, and individual electrodes are cut using a diamond saw. The electrode has negligible cross-talk or phase-shift distortion.

Supported by the Veterans’ Administration and by USPHS (NS18741).

490.5

Cross-correlations between firings of simultaneously recorded neurones that exhibit a strong periodic pattern are difficult to interpret in terms of the underlying functional connectivity. The evaluation of the state-dependent correlations for pairs of medial septal (MS) neurones in the rat during hippocampal theta rhythm (θ) and large irregular activity (LIA) is therefore ambiguous. Under the assumption that the firing properties of MS neurones are reflected in θ and LIA, a correction procedure based on the Joint Peri Stimulus Time Histogram (JPSH) can be applied. By interpreting θ and LIA as the “stimulus” for time-locked MS activity, the JPSH was applied to neurone pairs from the MS that were recorded simultaneously with θ and LIA. The correction for the cross-correlogram amounted from one to one-half of the peak value, thus substantially reducing the periodic components but not eliminating them. The procedure allowed a more reliable comparison between correlations under θ and LIA states. In addition it was concluded that rhythmic hippocampal field activity is only partially accounted for by periodic activity in MS neurones.

490.6

The relationship between hippocampal glucose metabolic activity and electroencephalographic patterns was analyzed by cell layer in Long-Evans rats following unilateral fimbria-fornix (ff) lesions. Animals were implanted with a unilateral hippocampal recording electrode and baseline hippocampal theta activity was measured. Animals were divided in 2 groups: ff-lesioned, and sham-lesioned f-f lesion. The lesion consisted of aspiration of the f-f and overlying neocortex ipsilateral to the recording electrode. At 4 weeks post-op, theta activity was recorded during treadmill walking. After 3 months, each rat was prepared for measurement of cerebral glucose metabolic activity according to the 14C2-deoxyglucose protocol of Sokoloff, et al., 1977. Autoradiographs were superimposed on nissl sections with an Amersham imaging system and laminar glucose utilization was quantified. Analysis of hippocampi that sustained a post-lesion theta loss and those that displayed an eventual theta return demonstrates a correlation between hippocampal glucose metabolism and the presence of theta activity in hippocampal EEG. The pattern of glucose utilization only in the stratum oriens of the hippocampal CA1 region correlates with ipsilateral theta activity.

490.7
DETECTION OF AN ATROPINE-RESISTANT COMPONENT OF THE HIPPOCAMPAL THETA RHYTHM IN URETHANE ANESTHETIZED RATS. Steven E. Fox and Mark Stewart, Dept. Physiology, SUNY Health Sci. Ctr., Brooklyn, N.Y., 11203.

An important pharmacological feature of the hippocampal theta rhythm in urethane anesthetized animals is its atropine sensitivity to antimuscarinic drugs. This sensitivity may be partly due to a masking of the theta frequency by increases in both higher and lower frequencies. Some studies suggest that this component is resistant to theta rhythm. The discovery of atropine-resistant, rhythmic medial septal neurones has provided a physiological trigger for averaging EEG and unit activity after large atropine doses. Such averaging has permitted the detection of an atropine-resistant component of the hippocampal theta rhythm in urethane anesthetized rats. The post-atropine theta activity recorded from both CA1 (superficial to the pyramidal cell layer) and dentate (near the hippocampal fissure) in 15 rats was analyzed by cross correlation. Comparisons from the two locations maintained their phase relations to the septal units and to each other. The presence of this residual theta component after doses as large as 10 mg/kg indicates that it cannot be mediated by muscarinic cholinergic receptors. The coupling of the signal to the atropine-resistant septal cells strengthens our previous suggestion that these septo-hippocampal neurones are not cholinergic, and are therefore probably GABAergic. (Supported by NIH grants NS17095 and NS07117.)

490.8
MONKEYS HAVE HIPPOCAMPAL THETA ACTIVITY. Mark Stewart and Steven E. Fox, Dept. Physiology, SUNY Health Sci. Ctr., Brooklyn, N.Y., 11203.

The hippocampal theta rhythm has been extensively studied in many sub-primates. Some studies have suggested that the theta rhythm is essential for the normal functioning of the hippocampus. Efforts to generalize these implications to humans has been unconvincing since no clear examples of primate theta rhythm have been reported. Varying the specific behavioral correlates for the theta rhythm across species suggested that monkeys anesthetized with urethane might be a more appropriate preparation for initial studies of the hippocampal EEG in primates. Three macaques received ketamine (6-8 mg/kg im) to permit cannulation of a superficial leg vein for urethane injection. Two squirrel monkeys were initially ether anesthetized to permit venous cannulation. Four of the 5 monkeys (the exception being a very old squirrel monkey) showed a theta rhythm. The frequency profiles at the theta frequency had maxima between the dentate granule cell layer and the CA1 pyramidal layer. Similarities with the theta rhythm of urethanized rats included: 1) sensitivity of the urethane-induced theta activity to muscarinic drugs; 2) its correlation with spontaneous movements during light anesthesia. Important differences were: 1) the frequency of this theta activity was 7-9 Hz compared to 4-5 Hz found in rats; 2) considerable spontaneous heath frequency EEG co-existed with the monkey theta activity; and 3) durations of bouts of theta activity in monkeys were shorter than in rats.

Primates generate hippocampal theta activity in a way that is homologous to that of rats. The similarities between the monkey and rat EEG emphasize the utility of sub-primates for developing methods for studying hippocampal theta EEG in man. (Supported by NIH grants NS17095 and NS07117.)
BEHAVIORAL CORRELATES AND CHOLINERGIC MANIPULATIONS OF HIPPOCAMPAL PHASIC LINEAR θ-ON CELLS. L. V. Colon, S. Hopseth, B. H. Bland, University of Calgary, Department of Psychology, Behavioral Neuroscience Research Group, Calgary, Alberta T2N 1N4.

The firing patterns of phasic linear θ-on cells in the CA1 and dentate layers of the hippocampal formation of the freely moving rabbit were analyzed during 3 behavioral conditions: (1) voluntary motor patterns, termed type 1 θ behaviors; (2) automatic motor patterns, termed type 2 LIA behaviors; (3) alert behavior with presentation of sensory stimuli, termed type 2 θ behaviors. Phasic linear θ-on cells discharged rhythmically during type 1 and type 2 θ behaviors and increased their discharges in a linear positive manner in relation to increases in θ frequency. These same cells discharged irregularly and at lower mean rates during type 2 LIA behaviors. The discharge rates during type 1 θ behaviors were always greater than that occurring during type 2 θ behaviors, even at equivalent θ frequencies. Administration of ATSOq abolished the rhythmicity and linearity during type 2 θ behaviors. Cellular rhythmicity and linearity during type 1 θ behaviors was not affected by the drug but mean discharge rates were reduced in all 7 cells tested (x = 28.14 ± 18.02%; range = 5-56%).


The basolateral amygdala (BLA) projects richly to the ventral striatum (VS), but the behavioral functions of this “limbic-motor interface” remain unclear. In these experiments, selective, bilateral, axon-sparing lesions of the BLA and VS were made by infusing the excitotoxins, quinolinic and quinuclidinyl acetate, respectively. The effects of these lesions were investigated on the expression of (i) a conditioned place preference (CPP) acquired by prior pairings of a distinctive environment with 20% sucrose and (ii) a discriminated approach response in an operant chamber with sucrose reinforcement, in mildly deprived rats. Both BLA and VS lesions abolished the CPP, but differed in their effects on ingestive, locomotor and discriminative approach behavior. Lesions of the ventromedial, but not the dorsolateral, caudate-putamen also attenuated the CPP. Interactions between the BLA and VS were studied by unilaterally lesioning BLA together with the contralateral VS. This symmetric lesion also markedly attenuated CPP. These results strongly implicate amygdala-ventral striatal interactions in reward-related processes and endorse the functional importance of this limbic-motor interface.

DIFFERENTIAL EFFECTS OF SOCIAL CONTEXT UPON LATERALIZATION AND AMPLITUDE OF THE ELECTRICAL ACTIVITY OF THE AMYGDALA AND CORTEX IN SQUIRREL MONKEYS. B. L. Lloyd, A. S. Klug, F. Heagerty†, E. Mirzabekov†, and D. Riccit*. Psychiatry Service UCL/VA Medical Center, Sepulveda, CA 91343, and Biology Department, California State University, Northridge.

Electrical activity was recorded from amygdala and cortex in four animals seated in a restrainer chair, alone or with a conspecific. Power spectral analysis revealed an increase in total power relative to the amygdala in the animal in the presence of a conspecific, reflecting increases in the a, b, and d bands but not in t. No differences were observed from any combination of cortical electrodes.

In all frequency bands, except d, power in the right amygdala exceeded left in both conditions; d in the right hemisphere exceeded that of the left only when the monkey was in isolation. Cortical a and d were greater in the left hemisphere under these conditions. These results suggest a right bias in the processing of social/sensory information.

This study was supported by a grant from the Veterans Administration.

EFFECTS OF HIPPOCAMPAL LESIONS ON MACAQUE PERFORMANCE OF A TASK REQUIRING ISOMORPHIC SPATIAL MAPPINGS. B.D. Moss, P. Aranega-Rayes, C. Coley*, H. Pasquier, and G.C. Baylis. Dept. of Neuroscience and Psychology, University of California, San Diego, La Jolla, CA 92030.

The hippocampus of the macaque has been strongly implicated in memory processes. Much of this research has centered around tasks such as Delayed Non Matching to Sample (DNMS). The Wisconsin General Test Apparatus that we use is designed to avoid the confounds found in the DNMS, and the task is now very similar to the tests used with rodents. However, even at equivalent θ frequencies. Administration of ATSOq abolished the rhythmicity and linearity during type 2 θ behaviors. Cellular rhythmicity and linearity during type 1 θ behaviors was not affected by the drug but mean discharge rates were reduced in all 7 cells tested (x = 28.14 ± 18.02%; range = 5-56%).


The basolateral amygdala (BLA) projects richly to the ventral striatum (VS), but the behavioral functions of this “limbic-motor interface” remain unclear. In these experiments, selective, bilateral, axon-sparing lesions of the BLA and VS were made by infusing the excitotoxins, quinolinic and quinuclidinyl acetate, respectively. The effects of these lesions were investigated on the expression of (i) a conditioned place preference (CPP) acquired by prior pairings of a distinctive environment with 20% sucrose and (ii) a discriminated approach response in an operant chamber with sucrose reinforcement, in mildly deprived rats. Both BLA and VS lesions abolished the CPP, but differed in their effects on ingestive, locomotor and discriminative approach behavior. Lesions of the ventromedial, but not the dorsolateral, caudate-putamen also attenuated the CPP. Interactions between the BLA and VS were studied by unilaterally lesioning BLA together with the contralateral VS. This symmetric lesion also markedly attenuated CPP. These results strongly implicate amygdala-ventral striatal interactions in reward-related processes and endorse the functional importance of this limbic-motor interface.

DIFFERENTIAL EFFECTS OF SOCIAL CONTEXT UPON LATERALIZATION AND AMPLITUDE OF THE ELECTRICAL ACTIVITY OF THE AMYGDALA AND CORTEX IN SQUIRREL MONKEYS. B. L. Lloyd, A. S. Klug, F. Heagerty†, E. Mirzabekov†, and D. Riccit*. Psychiatry Service UCL/VA Medical Center, Sepulveda, CA 91343, and Biology Department, California State University, Northridge.

Electrical activity was recorded from amygdala and cortex in four animals seated in a restrainer chair, alone or with a conspecific. Power spectral analysis revealed an increase in total power relative to the amygdala in the animal in the presence of a conspecific, reflecting increases in the a, b, and d bands but not in t. No differences were observed from any combination of cortical electrodes.

In all frequency bands, except d, power in the right amygdala exceeded left in both conditions; d in the right hemisphere exceeded that of the left only when the monkey was in isolation. Cortical a and d were greater in the left hemisphere under these conditions. These results suggest a right bias in the processing of social/sensory information.

This study was supported by a grant from the Veterans Administration.


Rats received bilateral lesions of the basolateral amygdala (BLA) by infusing either quinolinic acid (QUIN) or 6-hydroxydopamine (6-OHDA). Lesioned and sham-operated rats were water deprived and pre-exposed to a two chamber, fixed ratio 1, 10 sec footshocks, to establish a reference condition. Immediately following pre-exposure, each rat was encosed in one chamber, where it experienced five pairings of a 30 sec, clicker CS and a 0.5 sec, 0.5 mA footshock. Half of the control group and half each lesion group were then lesioned with a 10 sec trace, and half with a 30 sec trace, interval between CS offset and shock onset. All rats were then re-exposed to the CS in a separate, operant chamber. Suppression of licking was used as a measure of fear conditioning to the CS, while preference for the safe side of the training apparatus measured fear conditioning to contextual cues. Consistent with the predictions of attentional theory, control rats in the 10 sec trace group had a normal deficit that was significantly greater than that of rats with trace conditioning to contextual cues.

Intra-accumbens dopamine (AMPH) enhances the effects of reward-related stimuli, as shown using an acquisition of a new response procedure with conditioned reinforcement (CR). A similar paradigm has been used to assess dopaminergic specificity of these effects. thirsty rats received pairings of water and a light/noise compound stimulus (CR) prior to a test phase in the absence of water in which responses were rewarded by a novel lever produced CR, but on the other had no effect. Intra-accumbens dopamine (5-50ug) or the D1 receptor agonist SKF38393 (0.01-10ug) dose-dependently and selectively increased responding on the CR lever, but the D2 agonist agonist LY171555 (0.01-10ug) was without effect. Noradrenaline (25-100ug) infused into the nucleus accumbens also did not affect responding for CR. The effects of intra-accumbens AMPH (18ug) were completely blocked by immediately antecedent intra-accumbens infusion of the D1 receptor antagonist SCH23390 (1ug) or the D2 receptor antagonist raclopride (5ug). These results show that both D1 and D2 receptors contribute to the effects of intra-accumbens AMPH, but that stimulation of D2 receptors in the nucleus accumbens is, by itself, insufficient to enhance the effects of CR.

490.16 HIPPOCAMPAL MODULATION OF MESOLIMBIC FUNCTION. T.L. Steele & L.D. Devenport. Department of Psychology, Unv. of Oklahoma, Norman, OK 73019.

Hungry rats with lesions of the hippocampal formation are stereotypic and hyperactive when exposed to cues associated with reward (Science, 1981, 212, 1280). This assumption was tested behaviorally by placing food-deprived sham and hippocampal lesion rats in an environment predictive of reward. As incentive motivation was intensified by increasing deprivation, sham rats became hyperactive and engaged in stereotypic behaviors identical to those displayed by hippocampal rats. Stereotypy, however, did not exceed levels commonly associated with mesolimbic DA activation in any of the animals. Based on these results, free-fed rats in a second study were given one of five doses of d-amphetamine (0.3 mg/kg) to determine if the lesion would selectively enhance low dose stereotypes while leaving high dose stereotypes unaffected. These expectations were confirmed. It is apparent that the hippocampus influences the mesolimbic system, while having little effect on behaviors associated with nigro-striatal activity.

490.17 DISTINCT TH AND DBH IMMUNOREACTIVE FIBERS IN THE MONKEY’S HIPPOCAMPUS. Y. Samson*, J.Wu*.

The distribution of TH and DBH-immunoreactive fibers was studied in the hippocampal formation (dentate, CA3, CA1, and subiculum) of Cynomolgus monkeys. Four hippocampal formations from two normal monkeys, and two from one monkey eleven days after bilateral fornix lesion were studied. In normal monkeys, the density of TH fibers was very high in the hilus and the molecular layer of the dentate gyrus; high in the 3 layers of the subiculum, and in stratum lacunosum-moleculare of CA1 and CA3. Only rare TH fibers were found in the remaining layers of CA1 and CA3, and in stratum granulosum of the dentate gyrus. By contrast, the density of DBH fibers was moderate or low, but DBH fibers were present in all areas except stratum granulosum of the dentate. In the animal studied after fornix lesion, a substantial decrease in both TH and DBH immunoactive fibres was limited to the rostral hippocampus. Since TH antiserum may preferentially label dopaminergic fibers, while DBH antiserum may label noradrenergic fibers, these findings suggest a moderate but widespread noradrenergic innervation of the monkey’s hippocampus, and a heavier, more restricted dopaminergic innervation. The loss of fibers after fornix lesion only in rostral hippocampus suggests that, as in rodents, these dopaminergic fibers may reach the hippocampus through at least two distinct pathways.

(Supported by the NIH (NS06233) and the VA).


We used silver degeneration staining (SDS) to assess the extent of neuronal damage in the rat hippocampus after insertion of dialysis probes. Probes were stereotactically placed in the hippocampus in Sprague-Dawley rats and the animals were sacrificed at specified intervals at varying intervals. Neuronal degeneration was evident in all animals. Cell body degeneration was usually apparent to a 2 to 3 cell layer immediately adjacent to the probe pathway. In addition, there was widespread argyrophilia of well defined anatomical pathways. Damage was present in the perforant path in CA1 and the dentate both proximal and distal to the implant, in the associational fibers in the stratum radiatum and stratum oriens in CA1 and CA3, adjacent to the probe, and occasionally in the commissural fibers of the contralateral CA1 region. When the probe penetrated region inferior, damage was present in CA3 mossy fibers and in Schaffer collaterals in both CA3 and CA1. While some damage is invariable when an object is implanted in the brain, both local damage and damage to fibers of passage were taken into consideration when the results of in-vivo dialysis are analyzed. (Sponsored by VA and DOD)


Previous studies in our laboratory have shown that induction of limbic seizures by systemic administration of kainic acid (KA) produced large increases in the concentration of kainic acid mRNA in limbic regions of the rat CNS (Kreutz et al., Neurosci. Abs., 13:1665, 1986). We investigated whether systemic KA administration produces an increase in the concentration of TH prohormone mRNA prior to the observed increases in TH concentration.

Male Sprague-Dawley rats (180-200g) received s.c. injections of KA (12 mg/kg in 0.9% NaCl) or vehicle. All rats were sacrificed 6 hours later and their brains rapidly frozen. 30μm sections were cut and affixed to gelatin-subbed slides. In situ hybridization studies were conducted using a [35S]dATP labeled synthetic oligonucleotide probe complementary to TH prohormone mRNA.

KA administration resulted in significant increases in TH prohormone mRNA expression were observed in the CA2 and CA3/CA4 pyramidal cell layers of the dorsal hippocampus of the bionomial, central, and medial amygdaloid nuclei. In addition, increases were observed in the dorsal and ventral endopiriform nuclei and in the peripirical and parolfactory cortices. TH prohormone mRNA in hippocampal hypotalamic nuclei was not altered following KA administration. These results indicate that the KA-induced regional increases in TH concentrations are preceded by an increase in TH prohormone mRNA expression.

491.2 NICOTINE INTERFERES WITH INHIBITORY PROCESSES IN MOUSE HIPPOCAMPUS. R.K. Freund, V. Lustig-Levyman* and A.G. Collins. Inst. for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

The excitatory effects of nicotine (Nic) in the hippocampus may be due to interference of GABA transmission (Freund et al. Brain Res., 533 (1988) 215). We predicted pulse experiments were used to learn whether Nic affects endogenous inhibitory systems in hippocampal slices from DBA/2 mice. Pairs of central CA1 pyramidal cells were driven orthodromically (O) to the Schaffer collateral fibers, such that both feed-forward and recurrent inhibitory fibers are activated by the conditioning pulse. In another series (AO), the O pulse was conditioned by a preceding antidromic (A) pulse to the alveus; this paradigm should activate only recurrent circuits. Normally, an A-conditioned PS is inhibited relative to the unconditioned response. For the AO experiments, Nic relieved this inhibition, or facilitated the conditioned PS, in a concentration-dependent manner (50-400 μM). For the AO experiments, Nic (200 μM) inhibited the conditioned PS. Nic may inhibit GABA transmission in either case, but the consequences of impaired GABA transmission depend on which fibers were used for conditioning.
DIHYDROALPRENOLOL (DHA) BINDING IN RAT BRAIN. C.A. 

This increase in binding as an increase in beta-adrenergic 

binding sites in rat brain. Previously, we interpreted

the increase in agonist-stimulated accumulation of cyclic AMP. What physiologi 

cal role of EAA-5HT interactions could play in memory reten 

tion opens questions for further investigations.

THE EFFECTS OF CARBACHOL ON EXTRACELLULARLY 

RECORDED RESPONSES IN THE RODENT DENTATE GYRUS 

MAINTAINED BY G. S. Kakher and C. W. Kehoe, Dept. of 

Psychobiology, University of California, Irvine, CA 92717.

Dentate gyrus granule cells receive excitatory amino acid neurotrans-

mitters from entorhinal cortex (perforant path) which terminate in the 

outer two-thirds of the molecular layer. This same region receives 

cholinergic input from the medial septum suggesting the possible 

interaction between these transmitter systems in the dentate gyrus. Indeed, applications of carbachol (a general acetylcholine agonist) reduce 

synaptic responses of dentate granule cells everted by perforant path 

stimulation. In this study, we investigated the effects of carbachol on 

extracellular field potentials (EFPs) recorded from rodent hippocampal slices 

maintained in vitro. Applications of carbachol reduced EFPs recorded from the middle third of the molecular layer, the terminal region of the 

medial perforant path (MPP), whereas responses recorded from the outer 

third of the molecular layer (lateral perforant path EFP) were not reduced.

Pharmacological studies suggest that carbachol-induced reduction of 

MPP EFPs is mediated by muscarinic acetylcholine receptors as indicated 

by atropine (a muscarinic specific antagonist) sensitivity. More detailed 

pharmacological studies indicate that this effect may be mediated specifically by the M3 muscarinic receptor subtype in the dentate. Co- 

applications of carbachol (1μM) and gallamine (an M3 preferring antagonist; 20μM) protect against carbachol-induced EFP reduction (75%), whereas co-applications of carbachol (1μM) and pirenzepine (an M3 preferring antagonist; 100nM) do not protect against this effect.

These results may be explained by the presence of a presynaptic inhibitory action of carbachol mediated by cholinergic M3 receptors localized in the MPP terminal region.

GLUCOCORTICOIDS SUPPRESS EXCITABILITY IN HIPPOCAMPUS. M. Jodik* and E.R. de Kloet, Div. of Molecular Neurobiology, Inst. of Molecular Biology and Medical Biotechnology, and Rudolf Magnus Inst., University of Utrecht, Utrecht, The Netherlands.

Pyramidal cells in the CA1 region of the rat hippocampus are a major target for adrenal corticosteroids as we investigated the effects of glucocorticoids on CA1 cells in hippocampal slices. The steroids were perfused for 20 to 90 minutes period of washout prior to recording. Such prior perfusion of slices from adrenalectomized (ADX) rats with 1 μM corticosterone or 1 nM 1-μM of the glucocorticoid against RU28362 markedly enhanced both the amplitude 

and duration of the afterhyperpolarization (AHP) induced in CA1 neurons by a 0.5 nA cadmium pulse (50 or 300 ms duration). The steroid effects on AHP were blocked by glucocorticoid RU28364. The action of the steroids is probably mediated by a postsynaptic mecha

nism since AHP were still affected by TTX (1 μM) and TEA (5 μM) were present. Preliminary observations indicate that the steroid prolonged the calcium-spike recorded under these conditions. We also found that the AHP in slices from ADX rats was significantly smaller than the AHP in slices from sham-controls, although no differences existed in overall resting membrane properties. While steroid hormones thus enhance the AHP, activation of the β-receptor by norepinephrine (NE) has been reported to diminish the AHP and spike accommodation. The latter effects of NE were more pronounced in slices from ADX rats than either in slices treated with steroids or in slices from sham-controls. Our data suggest that selective activation of the glucocorticoid receptor in CA1 cells enhances the AHP, possibly by a genomic action on calcium

channels. Both the direct effect of the glucocorticoids on the AHP and the effect on the NE-induced blockade of cell accommodation will result in a steroid-induced decrease of neuronal excitability in the hippocampus.

DETERMINATION OF A TIME-RESPONSE CURVE FOR ACUTELY 

INTUBATED ASAPITRNE IN FISHER 344 AND SPRAGUE-DAWLEY RATS. G.B. Freeman, J. Sobotta*, D. Hattan* Battelle Columbus Laboratories, Columbus, Ohio 43201 and NR, Washington, D.C., 20204

This study was the first in a series to define a rodent model to document the effects of amino acid-modulating compounds on central neurotransmitter function. A time response curve for a single oral dose of aspartame was determined in unfasted male Fischer and Sprague-Dawley rats. Regional brain concentrations of NE, DA, 5-HT and their metabolites were analyzed in the hypothalamus, cere

bellum, pons/medulla, hippocampus, striatum, cortex and midbrain/thalamus at 30, 60, 120 or 240 minutes after oral aspartame (1000 mg/kg) administration. The acute administra 

tion of aspartame had little effect on static levels of the catecholamines or indoles. No one strain had a greater or different sensitivity to aspartame than the other. Although the present study indicated that aspartame was without effect on monoamine metabolism, these data should be interpreted cautiously in view of the fact that data on blood and brain levels of the amino acids, phenylalanine and tyrosine, have not been determined as well as direct measurements of synthesis or turnover. In addition, the present study used unfasted rats which may have increased the possibility of aspartame binding to feed in the stomach. Several of these issues are being addressed in an experimental study currently in progress.

EFFECT OF SOCIAL CONFLICT ON BRAIN DOPA IN MICE. INTERACTIONS WITH NALTREXONE. A. Mawson* [1], K. Pederson [1], and B. Siegfried [2].


It has been shown that social conflict activates brain opioid systems in mice (Bulling et al, Brain Research 450:237-246, 1988). Moreover,

opioids are known to modulate dopamine (D) neurotransmission within the nigral-substernal and mesolimbic systems. In the present study, we investigated the effect of social conflict on brain D metabolism in BMA mice as well as the role of endogenous opioids in stress-induced changes in dopaminergic activity. Mice were pretreated with saline or the opiate antagonist naltrexone (2 g/kg i.p.). Ten minutes later, mice were confronted either to a nontreatment or to an aggressive opponent of the same strain. The aggression confrontation increased the levels of DOPC in hypothalamus, frontal cortex, and periaqueductal gray but had no effect in the striatum, olfactory bulb and olfactory cortex. The DOPC levels in DOPC were unaffected by naltrexone. In contrast, naltrexone blocked the increase in DA levels induced by aggressive confrontation in the frontal cortex and periaqueductal gray of BMA mice. The results further suggest that opioids mediates the social conflict-induced increases in DA synthesis in these brain regions.
941.9

Noradrenergic neuronal hyperactivity after chronic morphine administration has been suggested to be partially responsible for the signs and symptoms of opiate withdrawal. If the suppression of noradrenergic activity that occurs during morphine administration could be prevented, this might also prevent withdrawal effects. To test this hypothesis, we have continuously infused a low dose of yohimbine to morphine rats, in order to increase noradrenergic activity, during morphine treatment. Withdrewal was subsequently precipitated with naloxone. There were six groups: saline controls (N=11), morphine (N=11); morphine + yohimbine (2.5 mg/kg/day; N=11 or N=8), 2.5 mg/kg/day yohimbine (N=11 or N=5). Subjects received 75 mg morphine pellets or sham-pelleting, on day 1, 4 and 6 of the treatment. Yohimbine was delivered throughout the 28-day treatment by s.c. implanted pumps. On the third day, all subjects were given 1.0 mg/kg naloxone and ran for behavioral symptoms of withdrawal. Analgesia was also measured, by observing tail flick latencies (TFL) before treatment and before the test. Naloxone precipitated withdrawal only in those subjects that received morphine alone. Withdrawal was attenuated by the concurrent administration of yohimbine and morphine: Wet-dog shakes, teeth chattering, immobility, rhesmoresis and abnormal posture were significantly reduced compared with subjects treated with morphine alone. Although concurrent morphine and yohimbine treatment attenuated naloxone-precipitated withdrawal from morphine, analgesia measured by TFL was not attenuated. It is suggested that yohimbine-induced activation of the alpha-2 adrenergic receptors blocks withdrawal without reducing therapeutically useful opioid analgesia.

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941.11
INTERACTION BETWEEN TRACE AMINES WITH BIOGENIC AMINES REACTED IN SYNAPTOSOMAL MEMBRANE CHANGES. J. Harris, S. Trivedi and B.L. Rammohan. Chemistry Dept., Arizona State University, Tempe, AZ 85287-1604.

The question regarding a neurochemical role of "trace" amine neurotransmitters is explored using spin labelled lipid markers to follow changes in the organizational state (membrane fluidity) of isolated synaptosomes. Results revealed that the membrane fluidity induced by phenylethylamine (PE) or dopamine (DA), was completely dissolved with PE and DA with its agonist apomorphine (APO), which increased membrane disorganization more intensively than DA. In the same way, the fluidity of the membrane was unaltered from -28°C to 0°C but decreased the Ago effect on fluidity in the temperature range. The fluidity of the membrane was unaltered from -28°C to 0°C but decreased the Ago effect on fluidity in the temperature range. Peroxide (H2O2) and with tryptamine (TRY), serotonin (5HT), but not veratridine, caused the membrane fluidity to increase. Therefore, it can be suggested that tryptamine and serotonin, but not veratridine, cause an increase in the membrane fluidity and not increase the release of specific neurotransmitter. These results support the hypothesis that trace amine neurotransmitters can be modulated by the receptor subtypes (Cell Mol. Neurobiology, 2: 87-94, 1989).

941.12
EFFECTS OF ORGANOPHOSPHATE INTOXICATION ON LEVELS OF CENTRAL TRANSMITTERS AND THEIR METABOLITES MEASURED WITH HPLC. M. El-Etr, W.T. Nickell and M.T. Shidy, Dept. of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Muscarnic receptors are known to regulate the release and possibly the metabolism of other central transmitters; thus, the symptoms of AChE inhibition by organophosphate poisoning may result partially from secondary effects of excitotoxicity on these other transmitters. We have measured changes in the levels of biogenic amines in the olfactory bulb and striatum produced by sub-lethal doses of the irreversible oxime, Malathion. Adult male Sprague-Dawley rats were injected i.m. with 0.75ED50 of soman in saline; control rats received the vehicle. Twenty-four hours following the injection, surviving animals were killed by decapitation. The brains were rapidly removed, the olfactory bulbs and striata were dissected, and samples were quickly frozen on dry ice. The levels of 3,4-dihydroxystyryl acid (DOPAC), homovanillic acid (HVA), dopamine, norepinephrine, 5-hydroxyindoleacetic acid (5-HIAA), and serotonin were determined by HPLC/EC.

The most significant changes produced by toman intoxication were an increase in HIAA, the major metabolite of serotonin, in the striatum, and a decrease in DOPAC and HVA, the major metabolites of dopamine, in both structures. These results suggest that excess ACh produced by AChE inhibition alters the metabolism and release of dopamine and serotonin in two forebrain structures. Thus, therapeutic approaches to organophosphate poisoning might be improved by treatment of these secondary effects. (Supported by DAMD 17-86-C-0005 and NS2348).

941.13
THE EFFECTS OF AMPHETAMINE AND Pilocarpine ON THE RELEASE OF ASCORBIC AND URIC ACID IN SEVERAL RAT BRAIN AREAS. A. Shiplev. Dept. of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Ascobic acid (AA) is present in high concentrations in brain. Because extracellular AA varies in response to synaptic transmission, AA may have a function in brain. However, most research on AA has been limited to striatum and to dopaminergic drugs. Even fewer data are available on uric acid (UA); extracellular UA also varies in response to neurotransmitter.

Linear sweep voltammetry was used to investigate the effects of amphetamine (which enhances the release of dopamine) and pilocarpine (which enhances the release of acetylcholine) on the release of AA and UA in brain areas differing in dopamine and acetylcholine concentrations. In caudate, nucleus accumbens, and hippocampus, the magnitude of the amphetamine-induced increase in AA was roughly correlated with dopamine content of the brain area tested. Cingulate cortex was more sensitive to pilocarpine. Pilocarpine produced the greatest increase in AA in cingulate cortex, even though cingulate cortex has the lowest acetylcholine concentration of the brain areas tested. The AA data were consistent with the hypothesis that amphetamine and pilocarpine release different pools of AA. The UA data were consistent with the hypothesis that amphetamine and pilocarpine release the same pool of UA.

Recent key studies have shed new light on the mechanisms by which melatonin affects CNS function to regulate a variety of processes including biological rhythms, neuroendocrine systems, sleep and retinal physiology. Dr. Takahashi will address the origins of CNS melatonin and in vitro studies on the cellular mechanisms controlling the circadian secretion of melatonin from the pineal and the retina. Dr. Dubocovich will discuss the identification and pharmacological characterization of neural receptors for melatonin using quantitative, in vitro functional and radioligand binding assays and the development of selective receptor agonists, including the first competitive antagonist luzindole. Dr. Reppert will present autoradiographic studies which visualize melatonin receptors in discrete CNS regions such as the suprachiasmatic nucleus (SCN), site of a putative biological clock, and the median eminence in adult and fetal brains from rodents and humans. Dr. Cassone will discuss data demonstrating that melatonin affects circadian rhythms in a number of species including man and will comment on the clinical implications of melatonin's entraining effect for treating circadian rhythm disorders such as jet lag, sleep disorders and depression.

VISUAL PSYCHOPHYSICS AND BEHAVIOR III

497.1 COLOR FILLING: PSYCHOPHYSICAL EVIDENCE AND A NEURAL NETWORK MODEL. M.A. Paradiso and K. Nakayama, Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115

If a person with a retinal scotoma has their scotoma surrounded by a visual target of a single color, that color will appear to fill-in the blind spot. Experiments with images stabilized on the retina of normal subjects demonstrate that in this special situation filling-in can also occur in people with intact visual systems. We will report the results of experiments which strongly suggest that even in normal (non-stabilized) vision there is a dynamic temporal filling-in mechanism underlying the perception of homogeneously colored regions. In one of our experiments a large disk-shaped target of a single color was briefly presented to an observer and shortly thereafter a white circular mask of smaller radius was presented. The mask was found to have a strong depressive effect on the perceived brightness of the center of the disk. Experiments in which the radius of the target and mask were varied suggest that color propagates from the edges of the homogeneously colored target. The mask appears to interrupt or interfere with this filling process. We have examined several different neural network models, some of which have been used by others to account for color vision, to determine whether they can account for our findings. The models have the common feature that color borders excite cells and this activation spreads across the network because of lateral excitatory connections. The models differ in the manner in which the color is bounded. For instance, lateral inhibition or shutting inhibition can effectively stop the spread of a color into inappropriate areas. We will present a shutting model which has been successful in accounting for our experimental results.


We present the first evidence that cats are able to discriminate fine orientation differences of illusory contours. Two types of illusory contour have been used (after Vogels and Orban, Vision Res., 27:63, 1987). For both illusory contour types, 30 daily sessions sufficed to train each of 2 cats to threshold for 2 reference orientations (horizontal and right oblique). Thresholds were measured using a 73.5% correct Wetherill and Levitt staircase as well as the method of constant stimuli and were identical for both reference orientations. The magnitude of the thresholds was lowest (10-13 degrees) if 3 or 4 pairs of inducing circle halves constituted the illusory contour. Both increasing or decreasing this number deteriorated discrimination performance. When only the outer pair of inducing circle halves was present, no reliable thresholds could be measured, suggesting that local cues are insufficient for the cat to solve the discrimination problem. This was confirmed in a control experiment, showing increasing thresholds as a function of random rotation over increasing angles of all pairs of inducing circle halves. As in humans, orientation discrimination thresholds obtained with illusory contours are elevated compared to those obtained using real lines (less than a factor 2 in humans, a factor 2-3 in cats).


It is established that either areas 17 or 18 are required for cats to achieve fine orientation discriminations. If a person with a retinal scotoma has their scotoma surrounded by a visual target of a single color, that color will appear to fill-in the blind spot. Experiments with images stabilized on the retina of normal subjects demonstrate that in this special situation filling-in can also occur in people with intact visual systems. We will report the results of experiments which strongly suggest that even in normal (non-stabilized) vision there is a dynamic temporal filling-in mechanism underlying the perception of homogeneously colored regions. In one of our experiments a large disk-shaped target of a single color was briefly presented to an observer and shortly thereafter a white circular mask of smaller radius was presented. The mask was found to have a strong depressive effect on the perceived brightness of the center of the disk. Experiments in which the radius of the target and mask were varied suggest that color propagates from the edges of the homogeneously colored target. The mask appears to interrupt or interfere with this filling process. We have examined several different neural network models, some of which have been used by others to account for color vision, to determine whether they can account for our findings. The models have the common feature that color borders excite cells and this activation spreads across the network because of lateral excitatory connections. The models differ in the manner in which the color is bounded. For instance, lateral inhibition or shutting inhibition can effectively stop the spread of a color into inappropriate areas. We will present a shutting model which has been successful in accounting for our experimental results.

497.4 LESIONS OF AREA 18 IN THE CAT REDUCE SENSITIVITY TO DRIFTING, LOW SPATIAL FREQUENCY TARGETS. Tatiana Pasternak, Marc Dorfman*, and John H.R. Maunsell, Dept of Neurobiology and Anatomy, Physiology, and Center for Visual Science, University of Rochester, Rochester, NY 14627

Psychophysical studies in cats and humans show that stimulus motion is discriminated best when the targets move briskly and are of low spatial frequency. Such targets provide optimal stimulation for neurons in cortical area 18 of the cat. To assess the contribution of this area to motion processing, we placed unilateral isotonic lesions in physiologically identified portions of area 18 in two cats. The lesions were centered in the representation of the lower right visual field, about 9 deg from the vertical meridian. We measured detectability of various spatiotemporal targets placed within the ablated and intact portions of the visual field representations, while monitoring eye position with a scleral search coil. The cats were required to maintain fixation on a laser spot and respond to the presence or the absence of a grating by pressing a right or left pedal. We found a nearly 10-fold loss of sensitivity to low spatial frequency (0.3 c/deg) gratings drifting at 4.5 Hz, placed within the ablated representation of the visual field. The sensitivity loss decreased at higher spatial frequencies and the resolution limit measured in this part of the visual field was identical to that measured in the intact hemifield. Since sensitivity for discriminating stimulus direction is maximal for low spatial frequency gratings, drifting at higher speeds, the inability to detect such targets after lesions of area 18 suggests that this cortical area represents an important stage in cortical processing of motion signals. (Supported by EY06175, EY0319)

The magnocellular retino-geniculate pathway comprises only about 10% of retinal ganglion cells, but it provides the major input to impor-
tant cortical structures. Its cells have large dendritic fields and large axons, and physiologically they show broad-band response to wave-lengths. Magnocelluar afferents play important roles in the high-temporal frequencies. In this study, we examined the role of this pathway in vision by making intracellular injections in magnocellular layers of the lateral geniculate nucleus and then monitoring the firing thresholds in por-
tions of the visual field corresponding to the resulting lesions.

Lesions at 6 deg eccentricity along the horizontal meridian did not reduce contrast sensitivity for the detection of moderate spatial frequency (2 c/deg) gratings presented either without modulation, or with 10 Hz counterphase modulation. However, such lesions did reduce fission resolution, and the magnitude of this effect was greater at lower contrasts. The stimuli in the latter experiment contained higher tem-
poral and lower spatial frequencies than the 2 c/deg - 10 Hz stimuli for which no loss was found. This result suggests that contrast thresh-
holds may be mediated by the magnocellular pathway at either high temporal or low spatial frequencies. This implication, as well as the role of this pathway in visual discrimination, are now under study.

Supported by grants AFOSR, ES01247, and EY0139.

497.7 COMPUTER SIMULATIONS OF MULTIPLE, INTERCONNECTED VISUAL CORTEX AREAS: FUNCTIONAL INTEGRATION AND ILLUSORY RESPONSES. I.L. Pankin. The Neurosciences Institute and The Rockefeller University, New York, NY 10021.

A large-scale computer simulation of the magnocellular pathway in areas VI, V3, and V5 (MT) of macaque visual cortex has been used to study the mechanisms of integra-
tion of distributed cortical networks. The simulated net-
works contain over 220,000 neuronal units and 8 million connections. Physiological properties of units in each area correspond to those observed in vivo, including direction selectivity in simulated area V5 similar to that described by Movshon et al. (Exp. Brain Res. Suppl. 11:117, 1985).

Visual stimuli are presented and network responses are compared to reported physiological and psychophysical observations. The integrative actions of the system are most clearly revealed by responses to several visual illusions including illusory contours (IC's), structure-from-motion (SFM), and a novel combined illusion which uses SFM to generate illusory contours. The same network architecture discriminates occlusion boundaries, IC's, and SFM by performing the same neuronal operations on inputs from different areas.

The simulations provide a framework for studying possible mechanisms of cortical integration and generate a series of testable experimental predictions.


This experiment investigates the role of relative position information in guid-
ging saccades to remembered targets. Stimuli were presented in the dark on an oscilloscope with a P31 phosphor, viewed through a long wave cut-off filter to minimize persistence. Subjects maintained fixation while two test targets were presen-
ted simultaneously. The test targets were extinguished and after a dark interval the target to be naged reappeared in one of the two previously presented test locations. This cued subjects to make a saccade first to the center of the visible target, and then to the center of the previously displayed memory target. In a second condition, the target to be naged reappeared after presentation of the memory target. In the first condition, the spatial rela-
tionship between the two targets is made available. In the second condition it must be computed from the information made available in the interstimulus interval. In both conditions targeting accuracy was good compared with visual targets. However, performance was substantially better in the simultaneous condition. This result suggests that the spatial relationship between simultaneously appearing targets is stored in memory and is used to improve the accuracy of sac-
cades to targets when they are no longer visible. In a second experiment, an extra saccade was introduced between target presentation and the trigger. This had little effect on error in either simultaneous or sequential condition. This suggests that unmoni-
tored drift in the dark intervals is the only additional source of error in the sequential condition.

Supported by EY01319, EY00729, and EY07344.

497.6 EFFECTS OF INTERTEMPORAL CORTEX LESIONS ON PATTER

FROM-MOTION DISCRIMINATION IN MONKEYS. K.H. Brittain*, W. T.

Newcombe, and R.C. Saunders. Stanford University and NIMH.

It is known that removal of inferotemporal (IT) cortex causes permanent pattern discrimination deficits in monkeys when the patterns are defined by luminance cues. Such deficits are observed in retention of preoperatively learned discriminations and in the recognition of new patterns. It is not known whether such deficits obtain when the patterns are defined solely by relative motion cues. This question is of interest since visual cortical areas that are specialized for the analysis of motion project chiefly to IT cortex and less to temporal cortex. This issue was addressed by comparing the performance of monkeys with IT lesions against that of paired controls on 2D pattern-motion discrimination problems. The pattern-from-motion task consisted of high-density, high-contrast random dot patterns presented within 2 circular apertures on a CRT screen. Each aperture contained a single geometrical figure within which the dots moved antiparallel to those in the surround. The monkeys received a liquid reward for selecting the "positive" pattern in each pair. The monkey indicated its choice by fixation for 2 seconds. After achieving criterion performance on 2 pattern-from-motion and 2 pattern-from-luminance problems, 3 monkeys received bilateral ablation of areas TE and TEO. As expected, the animals with IT lesions had severe deficits of both retention and acquisition of luminance-based discriminations. For pattern-from-motion discriminations, retention of preoperatively learned problems was impaired, but postoperative acquisition was unaffected. From the retention results, it would be inferred that IT cortex is involved in pattern-from-motion discrimination, which is not the case for the learning of pattern-from-luminance. (Supported by NEI grant EY5603).

497.8 VISUAL MOTION ANALYSIS WITH IMPAIRED SPEED PERCEPTION: PSY-


Over the past several years we have studied numerous patients with specific psychophysical deficits of motion analysis due to local lesions to the visual cortex. Lesions were unilateral and were localized by CT studies. Here we report results from a series of patients with lesions to unilateral lesions involving the visual cortex. First, we assessed performance on tasks involving color, form, binocular stereopsis, contrast, texture and motion discrimination. Secondly, we investigated ability to detect relative motion and from relative motion, to recover 3-D structure from motion, and to perceive motion coherence. To minimize position cues, we used dynamic random dot displays, varying dot density. Patients' performance suggests that (a) contrast sensitivity and discrimination of form, color, or texture can be decoupled from perception of speed, (b) perception of speed of motion can be decoupled from ability to recover structure from motion and from ability to perceive coherent global motion from random local motion fields; and (c) motion may be used for perception of speed. This finding suggests that information concerned with motion processing is not critical for perception of visual motion. These subjects were impaired on speed discrimination and perception of motion coherence. Stereopsis was lost in both patients. They were able, however, to detect 3-D structure from motion, and contrast, form, and color discrimination were normal.

498.10 ORIENTING TO NEGLECTED HEMISPHERE DEPENDS ON THE GEOMETRY AND SALIENCE OF THE DISPLAY. M. Rizzo and R. Burtt. Div. of Behavioral Neurology and Cognitive Neuroscience, Univ. of Iowa College of Med., Iowa City, Iowa 52242.

Humans with unilateral cerebral lesions may fail to explore contralateral visual hemispace. We had an opportunity to observe how such deficits may depend on properties of the stimulus under consideration.

We studied 3 subjects with CT/ME verified unilateral hemisphere lesions. Hemineglect was evident on tasks such as line cancellation. Eye movements were recorded using EOG (DC coupled), bandwidth 0-35 Hz, resolution 0.5 deg. The subjects showed deficient tracking of superthreshold light targets into the affected hemispace; one subject completely failed to follow any target directed to the left. When we present-
ed visual scanning of picture scenes and faces perform-
ance was improved. Recruitment of fixation into the abnormally fixated hemifield depended on feature density, con-
tinuity, and stimulus salience. Faces, which are frequently encountered objects of high signal value, were more likely to engage scanning across the midline. Results suggest that visual orienting into neglected hemispace depends on: (1) bottom-up factors related to geometrical closure, fine struct-
urals, and conjunctions among contiguous features, and (2) top-
down factors related to previous knowledge or biological salience of target displays.

In the present study we asked if a synthetic tube guide without any peripheral nerve components allows primate nerve to regrow over a long distance. Under deep nembutal anesthesia, the tibial nerves were sterilically exposed at the thigh and transected twice to remove a 2.0-2.5 cm nerve segment from adult monkeys (Macaca cyclopis). The resulted nerve stumps were anchored with a 9-0 prolene stitch in silicone tubes (11FR, Goleta, CA) to maintain a 3cm or 4cm interstump gap. Five weeks after surgery, there was a nerve cable connecting the nerve stumps. At twelve weeks numerous nerve fascicles containing many unmyelinated axons and few myelinated axons were seen at the mid of the cable and the distal end of the tube. We therefore conclude that regenerating axons are able to grow across a 4cm distance without an aid of autologous nerve graft in monkeys. (Supported by the American Muscular Dystrophy Association, CMRP260 of Chang Gung Med. Coll. and NSC78-0412-B182-11 of R.O.C.)

REGENERATION II

NERVE REGENERATION THROUGH SILICONE TUBES IN ADULT DORSAL AND VENTRAL ROOT AXONS IN SEMIPERMEABLE GUIDANCE CHANNELS. M. McCormack*, V. Gutrand, M. Goddart*, A. Beaurage*, S. Brace, P. Archibee, SPON, K. Thring, Artificial Organ Laboratory, Brown University, Providence, RI 02912.

The use of semipermeable guidance channels has been reported to provide a more appropriate regenerating environment by allowing solution exchange across the channel's wall. In the present study we evaluate the ability of semipermeable guidance channel to support regeneration of both dorsal and ventral roots. In one cohort the L5 dorsal root (n=3) was transected, a 2 mm segment excized and an entubulation repair with a 1 pm pore opening in silicone tubes (11FR, Goleta, CA) to maintain a 3 cm interstump gap. Following a 6 month implantation period, HRP labeling of dorsal root lesions showed no regrowth of dorsal root fibers into the rat spinal cord. The nerve stumps were serially monitored over 40 weeks following surgery. Changes in high variance smiles were easier to detect than changes in ones with low variance. Contrast sensitivity functions can determine the relative detectability of expressions by different individuals in different contexts.

Changes in high variance smiles were easier to detect than changes in ones with low variance. Contrast sensitivity functions can determine the relative detectability of expressions by different individuals in different contexts.

Neurophysiological experiments with monkey facial expressions can explore whether contrast sensitivity functions predict neural firing rates. Detection accuracy values greater than predicted by the objective measures of variance and entropy might indicate activation of neural feature detectors selective for facial expression.

REGENERATION II

COMBINED USE OF EMBRYONIC NEURAL TISSUE AND SYNTHETIC POLYMER TUBES TO PROMOTE CNS AXON REGENERATION. M. Klouty A. Kader, R. Risolo P. Britto, P. G. Facundo and J. Silver* Neurological Institute, NYC, NY 10032 and Case Western Reserve Univ. School of Medicine, Cleveland, Ohio 44106.

In the present study we asked if a synthetic tube guide without any peripheral nerve components allows primate nerve to regrow over a long distance. Under deep nembutal anesthesia, the tibial nerves were sterilically exposed at the thigh and transected twice to remove a 2.0-2.5 cm nerve segment from adult monkeys (Macaca cyclopis). The resulted nerve stumps were anchored with a 9-0 prolene stitch in silicone tubes (11FR, Goleta, CA) to maintain a 3cm or 4cm interstump gap. Five weeks after surgery, there was a nerve cable connecting the nerve stumps. At twelve weeks numerous nerve fascicles containing many unmyelinated axons and few myelinated axons were seen at the mid of the cable and the distal end of the tube. We therefore conclude that regenerating axons are able to grow across a 4cm distance without an aid of autologous nerve graft in monkeys. (Supported by the American Muscular Dystrophy Association, CMRP260 of Chang Gung Med. Coll. and NSC78-0412-B182-11 of R.O.C.)

REGENERATION II

REGENERATION II

REGENERATION II

REGENERATION II

FIBERS AND POLYMERS Lab., Mass. Inst. of Tech., Cambridge, MA 02139; Division of Neurology, Brigham and Women's Hospital, Boston, MA 02115; The New England Deaconess Hospital, Boston, MA 02115; and Division of Neurosurgery, Mass. General Hospital, Boston, MA 02115.

A continuing study of preferences of elongating axons and Schwann cells for specific matrix features has revealed two apparently critical requirements. Well-defined, chemical analogs of ECM based on a collagen-glycosaminoglycan (CG) copolymer were used to bridge a 10-mm gap between cut ends of the rat sciatic nerve. The nerve stumps and the CG matrix bridging them were ensheathed in a silicone tube. Electrophysiological properties of regenerating motoneuronal fibers innervating the plantar flexor muscles were serially monitored over 60 weeks following surgery. The results suggest that functional recovery of motor function requires the presence of a) a rapidly degrading CG copolymer matrix and b) an average pore diameter of order 1 um. These results pose novel questions about the nature of cell-ECM interactions during regeneration.

Supported by NSF Grant INT-8520548.
949.6 NERVE GROWTH FACTOR ENHANCES EARLY REGENERATION OF RAT MYELINATED AXONS THROUGH SILICONE CHAMBERS. K.M. Rich, J.C. Fryer, and J.P. Holloway, Departments of Neuropsychology and Neurobiology, Washington University Sch of Med. St. Louis, MO 63110.

The influence of NGF on regeneration of myelinated axons across a gap in adult rat sciatic nerve was examined to determine whether axons normally enhances sensory and/or motor axonal growth. Either dorsal root ganglioneuromas (DRG), or nerve segments were prepared in silicone chambers containing either 1 mg/ml NGF (experimental) or normal saline (control). Four weeks after surgery, semi-thin cross-sections of the implants stained with toluidine blue. Myelinated axonal counts were determined from both the proximal and distal ends within the chamber of the regenerated nerve. Thin sections were prepared for ultrastructural analysis. After the DRG, and the VR, there were mean counts of 2859 (motor) and 6794 (sensory) myelinated axons in the sciatic nerve proximal to the chamber in the respective groups. In the NGF-treated VR group within the chambers, there were proximately 4712 ± 808 and distally 1607 ± 368 sensory myelinated axons, compared to the control group with proximally 3236 ± 293 and distally 879 ± 238 sensory myelinated axons. In the NGF-treated DRG group, there were proximally 388 ± 92 and distally 77 ± 56 motor myelinated axons compared to the control group with proximally 327 ± 118 and distally 32 ± 19 motor myelinated axons. Although the motor myelinated axons did not grow across the chambers in significant numbers in either the NGF or control groups, ultrastructural examination demonstrated numerous unmyelinated axons in regenerative units in the distal portions of the DRG, chambers. Thus, NGF enhanced cholinergic axonal regeneration within a silicone chamber four weeks after nerve section. NGF had no effect on motoneuron regeneration in this experimental paradigm.


Adult mammalian CNS axons readily regenerate through peripheral nerve grafts containing viable Schwann cells but not well through whitewater, non-viable nerve grafts. One possibility is that living Schwann cells within the grafts contribute neurotrophic factors like nerve growth factor (NGF) which attract and promote elongation of incoming central axons. We compared CNS cholinergic axonal regeneration through freshly dissected peripheral nerve grafts with that occurring through "acellular" peripheral nerve grafts with that occurring through "acellular" PNS grafts, free of Schwann cells and debris, which had been treated with or without NGF. Acellular sciatic nerve grafts were prepared by 6 week pre-degeneration in situ, freeze-thawing, removal of macrophages and debris by incubation with deoxyribonucleic acid (DNA) and macrophages, freeze-thawing again and finally incubation with saline or saline plus purified 8-NGF: Two mm pieces of fresh autologous or acellular nerve were placed between bilaterally disconnected septum and hippocampal formation of adult female Sprague-Dawley rats. After one month, non-NGF treated acellular grafts contained a modest number of parallel AChE-positive fibers. NGF-treated grafts contained many more fibers and such fiber growth was comparable to that seen with fresh autologous control grafts. These results indicate that i) acellular peripheral nerve grafts can act as a barrier for CNS axonal regeneration and ii) treatment of the grafts with NGF before implantation allows them to perform as well as viable cellular nerve grafts. This suggests that, at least for CNS cholinergic axonal regeneration, Schwann cells in peripheral nerve grafts can be replaced by NGF. Supported by grants NIH NS-23011 and NSF BNS-84-08289.

949.10 SERINE PROTEASE INHIBITORS BLOCK POSTTRANSLATIONAL MODIFICATION OF PROTEINS BY ARG AND LYS. M. Yu*, G. Charraborty*, D. Luo and N. Ingoglia, Dept of Physiology, New Jersey Medical School, Newark, N.J.

The posttranslational incorporation of amino acids into proteins has been demonstrated in axons and supporting cells of a variety of neural tissues (Ingoglia et al., in Axonal Transport, Alan R. Liss Inc., 1987; 435). These reactions are regulated by endogenous molecules which must be removed from the reaction components in order for protein modifications to occur. In the present experiments we have attempted to characterize these molecules in brains and regenerating sciatic nerves of rats. Of a variety of exogenous substances examined, we have found that trypsin, a protease inhibitor, bovine pancreatic trypsin inhibitor (BPTI, MW = 6K) and alpha 2 macroglobulin are powerful blockers of these reactions, whereas inhibitors of calcium activated proteases, metalloenzymeproteases, thiol proteases and PMSF have little or no activity. Since the endogenous regulator of these reactions is a heat stable, 10-kd peptide which can be inactivated by trypsin, we propose that in vivo this is a serine protease inhibitor with BPTI-like characteristics. Supported by grants NS 19148 and E106782 from NIH.
Apolipoprotein (apo-) E produced by local macrophages and apo-A-I derived growth factor (PDGF) is involved in regulating the systemic regeneration. M. Lotan*, A. Cohen*, R. Puvdevani*

The visual system of fish and rat have been used as models for physiological significance of PDGF in the adult central nervous system following injury. We have now isolated and identified another protein produced by the cells of the injured rat sciatic nerve, apo-D. Apolipoprotein D has been identified previously only in plasma, where it is found on plasma lipoproteins. We have isolated apo-D from the lipoprotein particles present in regenerating rat sciatic nerves. It is not present, however, on the lipoprotein from rat plasma. We identified the nerve apolipoprotein as apo-D on the basis of amino acid sequence homology with human apo-D. Its amino acid composition, isoelectric point, molecular weight, and glycosylation pattern were also found to be similar to those of the human protein.

In the nervous system and glial cells, and that its production increases during regeneration. Western blot of extracts from regenerating sciatic nerves confirmed that apo-D increases in concentration severalfold during the first day following a crush injury, peaking at 3 weeks when it is increased 350-fold. Thus, neurons and glial cells responding to injury increase expression of apo-D, a protein that may participate in the lipid transfer processes of regeneration.

Calcinonin gene-related peptide (CGRP) is increased at the site of nerve injury. It is a likely candidate to have a role in the physiological aspects of regeneration. CGRP increases in concentration severalfold during the first day following a crush injury, peaking at 3 weeks when it is increased 350-fold. Thus, neurons and glial cells responding to injury increase expression of apo-D, a protein that may participate in the lipid transfer processes of regeneration.

Calcitonin gene-related peptide (CGRP) and its receptor are present in the central and peripheral nervous system. The release of CGRP from damaged sensory nerve terminals at the site of injury is considered to be involved in the processes of pain perception and peripheral nerve regeneration. The role of CGRP in nerve regeneration is still not fully understood, but it is believed to play a role in the process of axonal regeneration and nerve repair. CGRP is known to be a potent vasodilator and has been implicated in the regulation of axonal growth and nerve regeneration.

In conclusion we suggest that a) CGRP might be a mediator of pain, and b) CGRP might participate in the processes of pain perception and peripheral nerve regeneration. M. Lotan*, A. Cohen*, R. Puvdevani*


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499.3 The Effects of Calcium on Ganglioside-Mediated Neuritogenesis. P. L. Spooriti, A. K. Dolet*, C. G. Capila* and F. L. Holloman, Department of Neurological Sciences and Neurobiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

Considerable evidence suggests that gangliosides, especially GM1, play a role in the development of the nervous system. Previously, we have shown that exposure of murine Neuro-2a cells to GM1 resulted in dramatic neurite outgrowth. Furthermore, we have demonstrated that GM1 potentiated the action of NGF and NGF-independent trophic factors on a variety of primary neurons in vitro. The mechanism through which gangliosides exert their effects is unknown. To determine if ganglioside-neurite stimulation of GM1 neuritogenesis in Neuro-2a cells is due to altered intracellular calcium levels on GM1-mediated sprouting and elongation of Neuro-2a cells, these studies employed Ca2+ ion blockers (Ca2+EGTA, (+)-BML-151, and calcium adjusted medium). The result of GM1 neuritogenesis was quantitated microscopically with computer-assisted morphometry and ultrastructurally with electron microscopy. The neuritogenic activity of GM1 was enhanced by A213187, taurine and high extracellular Ca2+ and diminished by agents which reduce the intracellular Ca2+. We are employing x-ray microprobe analysis to localize Ca2+ in Neuro-2a cells after GM1 treatment. These studies suggest that the action of GM1 on Neuro-2a cells is Ca2+-dependent. USHS grants N24524 and D07734 to ESR.

499.4 Solubilized Muscle Membrane Fraction Regulates Motorneuron Adenylate Cyclase, F-Actin and ACh Sensitivity. J. Talbott Neuroscience, Univ. of Virginia Hlth Sci Ctr, Charlottesville, VA 22908.

Ciliary ganglion neurons from 11d avian embryos lose responses to acetylcholine (ACh) over 3-4 days of solitary cell culture, while neurons pharmacologically isolated in this period are capable of responding to acetylcholine. We have investigated the possibility that the loss of responsive neurons is related to the loss of ACh receptors from the plasma membrane. We have shown that the ACh receptor is a glycoprotein which is solubilized from the membranes of these neurons by treatment with Triton X-100, and is reinserted into a lipid bilayer with little thermodynamic loss. We have also shown that the solubilized ACh receptor can form a functional complex with the adenylate cyclase (AC) of these neurons. We have further shown that the ACh receptor of these neurons is sensitive to tyrosine kinase inhibitors, is found in a tyrosine-rich cytoskeletal region of the cell and is solubilized by a divalent cation (Ca2+, Mg2+) chelating agent. We have also shown that the ACh receptor is found mainly on the plasma membrane, but not the membrane of the cell nucleus. We have also shown that the ACh receptor is associated with a specific form of the ACh receptor, known to be a 67 kDa protein, which is found in the cytoskeleton. We have also shown that the ACh receptor is associated with a specific form of the ACh receptor, known to be a 67 kDa protein, which is found in the cytoskeleton. We have also shown that the ACh receptor is associated with a specific form of the ACh receptor, known to be a 67 kDa protein, which is found in the cytoskeleton. We have also shown that the ACh receptor is associated with a specific form of the ACh receptor, known to be a 67 kDa protein, which is found in the cytoskeleton.
499.9
REGULATION OF CELL ADHESION MOLECULE (CAM) LEVELS ON CULTURED RAT SCHWANN CELLS BY EPIDERMAL GROWTH FACTOR (EGF)

Recent studies have focused on the potential role in peripheral nerve regeneration, in particular on trophic interactions with regenerating neurons. Schwann cells have been shown to be involved in the regulation of levels of CAMs that are regulated by EGF. The present study shows that in vitro changes in EGF levels result in changes in CAM levels. Schwann cells of the second (Tr) guidepost at about the 33.5% stage. Although segment boundary

499.10
MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN DEVELOPING SYMPATHETIC NEURONS PROVIDED WITH EXCESS TROPHIC FACTOR SPACE
A.J. Boles, L. Concol, F. Schott, P. Remst, Wimmer* and T. Cianci*. Dept of Anatomy, Medical College of PA, Philadelphia, PA 19129

The pineal gland receives its innervation from sympathetic neurons located bilaterally in the superior cervical ganglion (SCG). In the present study, one SCG was surgically removed from newborn rats, and the animals were permitted to survive to adulthood. The pineal gland was operated on in this group of rats, and compared with the contralateral side. We observed that SCG neurons are capable of increasing their functional innervation to the pineal.

The cell bodies and the dendritic trees of these sympathetic neurons which had formed increased innervation in the contralateral pineal were examined by injecting the pineal with a conjugate of horseradish peroxidase and cholera toxin (HRP-CT). The cell bodies of the HRP-CT labelled neurons were larger than in controls, but the dendritic trees of these neurons were not enlarged. The number of branches and total dendritic length were decreased, while dendritic diameter was increased. Overall, the total volume of the dendritic trees was normal, and the total neuronal volume was increased.

These results indicate that an expansion of a neuron's axonal field is not necessarily accompanied by a concomitant expansion of its dendritic field. The increase in volume of the cell body that we observed may be due to a need by these neurons for enhanced production of neurotransmitter. (Supported by NS15952 and NS21822)

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500.3
CELL-CELL INTERACTIONS DURING THE FORMATION OF A COMMISSURAL PATHWAY IN THE EMBRYONIC GRASSHOPPER. Paul Z. Myer* and Michael J. Rapant (SPON: P. Pragin). Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.
We want to understand why the growth cones of commissural neurones bypass an ipsilateral target to select a presumably equivalent, but more distant, contralateral target. One hypothesis that we are pursuing is that commissural neurones have their targets re-specified during axonal outgrowth, perhaps after reaching the midline, by specific cellular interactions. In the grasshopper, the posterior commissure is pioneered by an identifiable neuron called Q1. The Q1 growth cone grows over the ipsilateral MP1/dMP2 axon, which pioneers the longitudinal fascicle, and crosses the midline to turn and grow caudally along the contralateral MP1/dMP2 axon. During axonal outgrowth, Q1's growth cone contacts and dyecouples with only a small number of neurones. Ipsilaterally, Q1 contacts and couples to the MP1/dMP2 neurones and the anterior and posterior corner cells, which also extend growth cones along MP1/dMP2. At the midline, the Q1 growth cone contacts its contralateral homolog and a midline neurone, MP4, and also becomes dye-coupled to the contralateral MP1/dMP2 and corner cells.
We are ablating the cells that the Q1 growth cone contacts to identify those neurones that are required for the formation of a normal commissure. Preliminary results suggest that the Q1 growth cone requires the presence of its contralateral homolog in order to follow its normal course across the midline.
(Supported by NIH NS25387, the McKnight Foundation, and the National Cancer Institute.)

500.4
PERTURBATION OF AXON GROWTH IN THE GRASSHOPPER LIMP BUD BY CHROMOPHORE ASSISTED LASER INACTIVATION. H. Keshishian & D. G. Jap*. Dept. of Biology, Yale Univ., New Haven, CT & Dept. of Cellular and Developmental Biol., Harvard Univ., Cambridge, MA.
Chromophore assisted laser inactivation (CALI) (Jav, D.G. PNAS 85:5454-58, 1988) was used to study axonal growth and guidance in the grasshopper limb bud. This procedure inactivates single protein functions with a dye labeled antibody subjected to nanosecond pulses of laser light of a wavelength absorbed by the dye but not by cellular components. The dye targets laser energy to denature bound proteins, leaving others relatively unaffected. The Tt pioneer neurones are a cell pair whose tightly fasciculated axons project stereotypically to the CNS. In insects, HRP binds specifically to a sugar moeity of neuronal membrane glycoproteins including the fasciculins. Dye labeled anti-HPR was injected into 30% grasshopper embryos that were subjected to laser irradiation to inactivate these proteins. The progress of axonal growth was visualized after 24 hours of further incubation. We found a three fold increase of defasciculation relative to nonirradiated controls (n=80). Other controls included nonirradiated and irradiated embryos injected with either unlabelled anti-HPR or dye labeled BSA. Defasciculation occurred in about 1/3 of the CALI treated limb buds. Often axons separated for up to 100 micrometers before rejoining as they progressed proximally. We also found examples of aberrant axonal growth and guidance. These experiments suggest a role for one or more of the anti-HPR cross reactive antigens in axonal adhesion. This demonstrates the use of CALI in studying molecular events in neurodevelopment.

500.5
SOMA POSITION DETERMINES IDENTITY OF PRIMARY MOTONEURONS IN DEVELOPING ZEBRAFISH EMBRYOS. J.S. Eisen. Institute of Neuroscience, University of Oregon, Eugene OR 97403.
The axial region of the zebrafish trunk is divided into a segmentally repeated set of three primary motoneurons. The two features that allow each primary motoneuron to be identified, its soma position and the region of muscle it innervates, are always correlated. To learn whether soma position specifies the region of muscle a motoneuron will innervate, I transplanted identified motoneurons between embryos.
Transplanted cells were placed in their normal position or shifted to the position of another motoneuron. Young motoneurons without axons developed normal arborizations when transplanted to embryos of the same stage. A motoneuron with soma position was shifted developed an arbor appropriate for its new soma position, suggesting that its identity was not fixed prior to axogenesis. Older motoneurons with axons also formed arbors appropriate for their new soma positions, when transplanted into hosts 1-2 hrs younger. This result suggests that motoneuronal identity was not fixed even after axogenesis. However, motoneurons with axons failed to form arbor when transplanted into hosts of the same stage as the donor embryos. Young motoneurons transplanted into embryos 1-2 hrs older also failed to form arbor.
These results suggest that soma position determines motoneuronal identity by specifying which region of muscle a motoneuron will innervate. While motoneuronal identity appears plastic even after axogenesis, age-related environmental features may regulate the expression of that identity by limiting the ability of a motoneuron to arborize. Supported by NS25915 and BMI855146.

500.6
The growth cones of zebrafish secondary motoneuron follow pathways established by the axons of pioneering primary motoneurons. This observation suggests that growth cones of secondary motoneurons may require the axons of primary motoneurons for normal extension. To test this model, we ablated primary motoneurons prior to axogenesis by laser-irradiation, and examined subsequent axonal outgrowth of the secondary motoneurons. We found that the axons of secondary motoneurons had not extended along pathways lacking primary motoneuron axons at stages when secondary motoneurons in control segments had grown to the distal limits of these pathways. Thus, secondary motoneuron outgrowth is either delayed or arrested in the absence of primary motoneuron axons. These results support the hypothesis that the axons of primary motoneurons are important for normal axonal extension of secondary motoneurons. Supported by NS25916.

500.7
PRIMARY MOTONEURONS INSTRUCT MUSCLE ACETYLCOLINE RECEPTOR PLACEMENT, BUT RECEPTORS DON'T INSTRUCT MOTONEURONAL SYNAPSE PLACEMENT, IN LIVE ZEBRAFISH. D.W. Lo & M. Winterfield. Institute of Neuroscience, University of Oregon, Eugene OR 97403.
Inductive interactions between nerve and muscle are known to be important for the development of each cell type. We have investigated the role of these interactions in the specification of synaptic connections by watching directly the development of labelled acetylcholine (ACh) receptor clusters on muscle fibers during innervation by labelled primary motoneurons in live zebrafish embryos, and by selectively eliminating either the neurons or the receptors.
We found that prior to contact by motoneuronal growth cones, the muscles lacked clustered receptors. Within minutes after a growth cone first contacted a muscle fibre, ACh receptors clustered at the site of contact and remained clustered at the neuromuscular junction. In mutant zebrafish lacking ACh receptors motoneuronal development was normal; neuromuscular junctions were found on appropriate muscle fibres. However ablation of primary motoneurons prior to axogenesis had a profound effect on the expression of muscle receptors; causing them to cluster ectopically and much later than normal.
We conclude that, in the zebrafish embryo, the influence of the motoneuron is required for appropriate clustering of muscle ACh receptors, whereas ACh receptor mediated interactions are necessary for the establishment of specific synaptic connections by the primary motoneurons. Supported by NS21132 & GM07575 & HD24246.
A very prominent activity in growth cones is the backward or retrograde movement of waves in the actin matrix which corresponds with backward movements of actin. Video-enhanced DIC microscopy allows retrograde movement even within filopodia of cortical neuronal growth cones (embryonic mouse, E13). Surprisingly, we also observed rapid forward movement within filopodia (see Wayne et al., this issue). Within many filopodia, swellings move towards their tips at 0.5 μm/sec. Movements are often discontinuous and packets can reverse direction. Transport was not observed in all filopodia and could be an unusual phenomenon; however, in the best optical situations frequent forward movements of smaller packets were seen indicating the presence of more extensive movements. Microtubules were seen in filopodia stained within antitubulin antibody. Active forward movements suggest that forward propulsion could drive process extension. Similarly, retrograde movements could be fueled by packets transported forward. An active transport model for processes extension is proposed.

Stereological and morphometric approaches were used to determine the distribution of target and non-target cells in vitro, D.H. Baird.


Before afferents interact with target cells, axonal growth cones extend through tracts and the target region, encountering other neurons and non-neuronal cells throughout this trajectory. To understand how neurite extension and growth cone behavior is influenced by both target and non-target cells, we examined neurite extension and growth cone interactions in our model culture system. Brainstem sources of cerebellar afferents (pontine nuclei) for mossy fibers were co-cultured with dissociated, purified primary growth cone target (granule) neurons, target neurons and astroglia. Explant neurites from mouse were identified on monolayers of cells from rat with the monoclonal antibody M13 (gift of K. Lagenaur), which stains mouse neurons exclusively. Explanted neurites fasciculate on the polylysine substrate in the absence of cells, or when grown adjacent to cells. Fasciculation is much reduced when neurites associate with target or non-target cells. When neurite outgrowth is abundant on cellular monolayers, neurite length is diminished on target neurons as compared to non-target cells from cerebellum or hippocampus. When analyzed with video-enhanced DIC microscopy, behavior of individual afferent growth cones with various cell types correlates with the above growth patterns: rapid elongation and fasciculation when pontine neurites meet each other; temporary cessation of elongation and maintenance of cell-cell contacts when pontine growth cones meet targets. These results suggest that target neurone temporarly arrest neurite extension, while contact with non-target cells permits elongation.

INVERTEBRATE LEARNING AND BEHAVIOR III

OPERANT CONDITIONING CAN BE SIMULATED BY SMALL NETWORKS OF NEURON-LIKE ADAPTIVE ELEMENTS. D.A. Baxter, J.L. Raymond, D.V. Haemmon and L. Byrnes. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025.

Activity-dependent neuromodulation has been proposed as a cellular mechanism for classical conditioning in Aplysia. Previously, we developed a mathematical model of an Aplysia sensory neuron that reflects the subcellular processes underlying this form of associative learning. This single-cell model can simulate features of non-associative and classical conditioning (Gangit and Thompson 1987). In addition, mathematical models of small networks containing adaptive elements with this activity-dependent neuromodulation "learning rule" can simulate some higher-order features of classical conditioning (Glick and Thompson 1987; Byrne et al. 1988; Hawkins 1989). In the present study, we test the hypothesis that this learning rule can also simulate a more consistent pattern of behavior. We use a network that contains seven elements, two of which are adaptive elements with the associative learning rule. A central pattern generator (CPG) consisting of two spontaneously active and mutually inhibitory neurons drives the network between two possible output states, each state being determined by activity in one of the CPG elements. Each of the CPG neurons drives an adaptive element, which in turn drives a motor neuron to produce one of the output states. Feedback from that output state of the CPG which drives it, regulates the duration of that component's burst. Reinforcement is provided by a modulatory neuron that synapses onto both adaptive elements.

We simulated operant conditioning by activating the modulatory neuron whenever one selected output state occurred. This led to changes in the relative amount of time the network spent in each of the output states. These changes are analogous to changes seen in studies of operant conditioning. Thus, our simulations illustrate that an associative learning rule, activity-dependent neuromodulation can simulate features of a number of different types of learning, including operant conditioning. Supported by grant AFSOR 87-0274.

REGIONAL SPECIFICITY OF GLIAL-GUIDED NEURONAL MIGRATION: CEREBELLAR GRANULE NEURONS MIGRATE ON HIPPOCAMPAL ASTROGLIAL FIBERS IN VITRO. J.E. Gassett and M.E. Hatten. Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

In most cortical regions of the developing mammalian brain, radially oriented astroglial fibers provide the primary pathway for neuronal migration. To test the regional specificity of glial-guided neuronal migration, we "mixed and matched" neurons and astroglias purified from ventral and embryonic eye fragments (from either nasal and temporal or dorsal and ventral retinae) were grafted onto the same optic stalk of the unlabeled host. Even in the absence of other optic nerve fibers, these grafted cortical cells were able to find their correct target sites on the tectum. This indicates that positional cues not only on the eye but also on the tectum guide the formation of the topographic retinotectal map.

ACTIVITY-DEPENDENT BLOCK OF CENTRAL SENSORY CONDUCTION DURING INHIBITION OF TAILWITHDRAWAL REFLEx IN APLYSIA. A.L. Catherwood and E.L. Walters. Dept. of Physiology & Cell Biology, Univ. Texas Med. Sch, Houston, TX 77225.

Presynaptic inhibition of mechanosensory neurons may be a mechanism of tail shock-induced inhibition of siphon withdrawal (Mackey et al. 1987). Knowing that some sensory plasticity in Aplysia involves cell-wide modulation of signaling strength (Billy & Walters 1989), we predicted that behavioral inhibition might involve central conduction block in sensory axons. A train of test stimuli to nerve p9 was used to activate axons of tail sensory neurons, and elicited tail contractions. Intense shock of another nerve (p8) or tail pinch significantly inhibited contractions evoked by p9 test stimuli for at least 10 min. Stimuli of p9 decreased the number of p9-elicited spikes recorded in tail sensory neuron somata in 10 of 20 preparations. This finding suggests that presynaptic inhibition of axons of one sensory neuron can have significant effects on axons of neighboring sensory neurons.
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PAIN PATHWAYS


Single afferent units were recorded from dorsal rootlets which were driven by electrical stimulation of the sural nerve in anaesthetized rats. The other hindleg nerves were cut. Weak and noxious mechanical stimuli; radiant heat pulses and cold stimuli were used for searching receptive fields in the skin. 49/52 A beta and 15/18 A delta units were driven by mechanical stimulation from their receptive fields, half of the A delta units being high threshold mechanoreceptors. In contrast, only 26/50 C-fibres could be driven by skin stimulation, most of them being nociceptors. Conduction velocities and electrical thresholds of responsive and non-responding C-fibres were not significantly different. When we only considered experiments when the skin had not been manipulated too much (first two filamentary stimuli), significantly fewer C-fibres (6/21) were responsive (p=0.005). We conclude, that at least some of the unresponsive C-fibres are "silent" nociceptors recruited only when their receptive endings are sensitized by inflammatory processes.


Single and multi unit recordings were performed in pentobarbitonal-anaesthetized cats. Thalamic responses to electrical (vagus and splanchnic nerves; Y, S) and natural visceral (taste, chemosensory and neuraxial nociception; esophagus, bladder and/or colon distension) and somatic (touch, pinch, heat) stimuli were mapped in nuclei surrounding the ventral posterior medial (VPM) region.

For both nerves, the distribution of the response loci was similar for ipsi- and contralateral thalami. Of the sites with electrically elicited responses 22% (19/86) were found in the peri- and reticular zone of VPM and none in VPM proper; 53% were located in medial dorsal (MD), central medial (CM) and central lateral n. (CL); 16% were found in zona incerta (ZI). 29% of the recording sites were sparsely distributed ipsi- and contralateral thalami. Of the sites with electrically elicited responses 10 mA; n +10) does not produce inhibition of the complex EPSPs but also a modest (non-significant) increase in both the MONO and COMPLEX EPSPs. The effect of SOC on the COMPLEX was significantly different from that of 5HT (p<0.05). These results suggest that modulatory neurotransmitters can affect elements in the siphon withdrawal circuit differently. It will now be important to identify the different sites of action of these transmitters, and determine the degree to which their effects contribute to the multiple components of plasticity observed at both behavioral and synaptic levels.
502.3 RETROGRADE LABELING OF SPINAL CORD NEURONS THAT PROJECT TO NUCLEUS SUBMEDIUS IN THE RAT. D.J. Dado and G.J. Gazeld, Jr. Dept. of Cell Biol. and Neuroanat. Grad. Prog. in Neuroscience, Univ. of Minnesota, Minneapolis.

Previous studies using anterograde labeling in rats, cats and monkeys have shown spinal and trigeminal projections to the thalamic nucleus submedius (BSM). In the present study, spinal injections of Fast Green Fluor (FG) were centered in Sm in five rats. After 3-5 days, 18 identified spinal segments and medulau were sectioned. In alternate sections, an average of fewer than 100 labeled spinal cord neurons were counted in each rat. A total of only 2 neurons were labeled in the marginal zone in these five rats. The majority of labeled spinal neurons were in the deep dorsal horn, ipsilateral to the labeled neurons in the spinal cord were contrastral. In contrast to labeling in the cord, a total of 179 marginal zone neurons were labeled in nucleus caudalis in the five cases. Control injections of the same size (n = 3) in the ventrobasal complex did not label any more than 400 spinal cord neurons. A control injection into the posterior thalamic nucleus labeled more than 700 neurons including those in the marginal zone. These results indicate that the spinal cord projection to Sm in the rat is small and does not originate from marginal zone neurons.

Supported by NS29502 and DA07234.


Physiologically identified single nociceptive-specific primary afferent fibers innervating the tooth pulp were stained intra-axonally with HRP at the level of Sm. Both types of fibers were found to pass in and out of the interstitial nucleus of the trigeminal tract and to terminate in different portions of the trigeminal spinal nucleus. Tooth pulp terminal profiles in the interstitial nucleus contained numerous mitochondria, clear, round vesicles, and large, dense core vesicles.

Large, dense core vesicles were not visualized in any section, but were numerous when present. Both tooth pulp terminal profiles formed asymmetric presynaptic contacts on small dendritic profiles and spines heads, and were postsynaptic to axonal profiles. An occasional contact was identified on the soma of cells in the interstitial nucleus.

HTM-tongue terminal profiles contained numerous mitochondria and clear, round vesicles. No contact to tooth pulp, only a few large, dense core vesicles were identified. HTM-tongue membranes formed asymmetric presynaptic contacts on small dendritic profiles and were postsynaptic to axonal profiles. Ultrastructural characteristics of HTM-tongue terminal profiles, but not tooth pulp, are similar to those of the cat.


Animal pain models that parallel human models allow the study of pain and analgesia under conditions in which the human perception is known and provide a clinical relevance that is frequently questioned with such tests as rodent tail flick and hot plate reaction. Also, by using a painful stimulus tolerated by humans and allowing the control of stimuli that is customary for human subjects, the ethical treatment of the animals is better insured. A pain model frequently used in humans is the cold pressor test, which approximates clinical acute pain and produces a tonic pain for studying counterirritation. In the present study, we have developed a primate cold pressor test that parallels that used in humans.

One adult female macaque received a liquid reward for pressing a button positioned at the bottom of a pan of refrigerated water. The monkey received a reward every 5 s while depressing the button, but had to wait 90 s before initiating a new trial after releasing the button. The time the monkey kept its hand in the water (withdrawal latency) was compared for water temperatures of 0°C, 10°C, and 35°C.

The monkey’s withdrawal latency varied directly with water temperature (34 s at 0°C, 78 s at 10°C and 180 s at 35°C, p < 0.02). Further, withdrawal latency was influenced by motivational level, as is usually true for pain tolerance measures. At each water temperature, withdrawal latencies were longer during trials early in a session, when the monkey had not received fluids for 12 h, than at the end, when the monkey was satiated. Pain thresholds from two humans in a similar task were shorter than the monkey’s withdrawal latency (17.5 ± 0.3°C and 51.2 ± 0.3°C, but tolerance time was longer than that of the monkey (31 s ± 0°C and 218 ± 0°C).

The present data suggest that the cold pressor test can be used successfully in monkeys as a pain model for the assessment of analgesic treatments and the study of endogenous pain-modulatory pathways.


The present data suggest that the cold pressor test can be used successfully in monkeys as a pain model for the assessment of analgesic treatments and the study of endogenous pain-modulatory pathways.

Background. Opioid receptors have been demonstrated on human primary afferents, and there are conflicting findings concerning their role as pain modulators.

Method. In experiment 1 ten ml morphine hydrochloride (4%) or ten ml isonicotic saline were injected peripherally to the ulcerated wound. The pain threshold to argon laser stimulation was measured before injection and 5, 10, 15, 30, 45, 60, 90 and 120 min after injection. In experiment 2 the arm was exsanguinated. After inflation of the cuff around the upper arm, 40 ml morphine hydrochloride (0.02%) or 40 ml isonicotic saline were injected intravenously. The cuff was deflated after 30 min. The pain thresholds were measured before deflation of the cuff, 20, 25, and 30 min after injection, and 5, 10, 15, and 30 min after deflation of the cuff. Ten healthy volunteers participated in each experiment. Injections were administered to the dominant arm.

Results. In either of the experiments no significant differences in pain thresholds were observed between morphine and saline injections. After 30 min of ischemia, total analgesia to laser-induced pricking pain was obtained.

Conclusion. For the morphine concentrations considered in the present study, the opioid receptor does not contribute to modulation of pricking pain.


Following cholinergic denervation of the hippocampus, mald medial septal lesions (MSL), peripheral sympathetic fibers, originating from the superior cervical ganglion, grow into the hippocampus. Both muscarinic and nicotinic receptors have been linked to phosphoinositol (PI) hydrolysis, we attempt to define the functional integrity of the hippocampus after MSL and sympathetic denervation (SD) by measuring PI hydrolysis and mitochondrial activity in the presence of carbocloth and norepinephrine (NE)-stimulated PI hydrolysis. Thirty adult male rats underwent one of three surgical procedures: (1) sham surgery; (2) MSL; (3) MSL + SD.

Carbocloth was found to enhance PI hydrolysis over basal levels in all three groups in a dose-dependent fashion. With maximal stimulation (5 mM), PI hydrolysis was significantly higher in the MSL + SD group (13.3 ± 1.1) compared to both the CON (9.8 ± 1.2) and MSL + SD groups (11.4 ± 1.3). Similarly, NE was also found to enhance PI hydrolysis in a dose dependent manner; however, no differences were observed between the three groups. These results suggest that PI is capable of altering hippocampal functional integrity through cholinergic mechanisms.

502.8 MORPHINE APPLIED TO HUMAN PERIPHERAL NERVES DOES NOT CAUSE ANALGESIA TO CUTANEOUS PAIN STIMULATION. L. Arendt-Nielsen, P. Birierg*, and J. Berg Dahl*. Dept. of Medical Informatics, Aalborg University, DK-9200 Aalborg, and Dept. of Dermatology, Marselisborg Hospital, DK-8000 Aarhus, Denmark.

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Conclusion. For the morphine concentrations considered in the present study, the opioid receptor does not contribute to modulation of pricking pain.

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503.1


A complete genomic clone has been obtained for human GFAP, the major component of intermediate diameter filaments of mature astrocytes. The nucleic acid sequence obtained for the coding region and intervening introns shows considerable similarity to that previously determined for the mouse gene. The mRNA start site, determined by primer extension and probe protection experiments, is also similar to that found for the mouse. By contrast, the initiating AUG for human gfa, determined by a novel in vitro transcription and translation method, leads to a deduced amino terminal sequence for human GFAP that differs markedly from that predicted for the mouse. This discrepancy was resolved by discovering that the published mouse nucleic acid sequence has an incorrect additional base.

The region around the mouse start site contains sites similar to consensus sequences for binding of several transcription factors, including those for AP-2, the TATA factor, and the Box A factor. The simultaneous presence of the latter two sites is unusual, as the TATA sequence is commonly part of promoters utilizing RNA polymerase II (Pol II), while the Box A sequence is commonly used by Pol I. Our studies show that these elements may both independently direct proper initiation of human gfa transcription by Pol II, and that the sites likely interact synergistically.

503.2


 mec-3 is a homeobox-containing gene required for expression of differentiated characteristics of a set of touch receptor neurons of the nematode Caenorhabditis elegans (Way and Chalfie, Cell 24, 5). To identify the cells in which mec-3 is expressed, we constructed a translational fusion between the mec-3 homeobox and lacZ, and injected it into C. elegans.

When transformed embryos are stained with X-gal the touch receptors are stained, as predicted. In addition, the FLP and PVD neurons also stain. We identified these cells by the pattern of DAPI-stained nuclei in X-gal-stained animals, and by the absence of stained cells in the unc-66 mutant, which lacks these cells due to lineage alterations.

The PVD neurons appear to be mechanoreceptors that sense a harsher touch stimulus. Elimination of these cells by either mutation or laser ablation abolishes the response to prodding in the center of the body (assayed in animals that lack the touch receptors).

The pattern of expression of the mec-3-lacZ fusion in mutant strains indicates that the homeobox-containing gene mec-3 is necessary for all mec-3 expression, and that mec-3 turns on its own synthesis.

503.3

HERPES SIMPLEX VIRUS TYPE 1 LATENCY-ASSOCIATED TRANSCRIPT EXPRESSION IN VITRO. W. Willinghagen* and B. Wiegand (SPON: R. Ziegler), Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Herpes simplex virus type 1 (HSV-1) is maintained in a latent form in neurons of the peripheral sensory ganglia. Fraser and coworkers (J. Virol. 54:849-852, 1985) have demonstrated that during latent infection of neurons the HSV-1 genome is most likely exists as a unit-length circular molecule. Studies by a number of investigators have shown that, if any, virus genes are expressed during latency. However, hybridization of RNA isolated from mouse superior cervical ganglia have resulted in the detection of a viral RNA species that accumulates throughout the course of latent infection. This transcript, termed the latency-associated transcript (LAT), accumulates to high levels in nuclei of latently infected neurons (Stevens et al., Science 235:1056-1058, 1987). Although dispensable for the establishment of latency, HSV-1 LAT may be required for the stable, long-term maintenance of latency (David et al., Virology 165:254-257, 1988). To examine the differential expression of HSV-1 LAT in vitro we subcloned the DNA sequences upstream from the HSV-1 transcriptional start site. Hind III linkers were added to the cloned fragments and the latter were transfected into a pG35 CAV A expression vector system for use in transient expression assays in a series of human and mouse neuroblastoma, glioblastoma, and fibroblastic cells. Because HSV-1 LAT is preferentially expressed at high levels only in latently infected neurons, it is reasonable to hypothesize that the viral RNA species which accumulates throughout the course of latent infection is intracellular receptor for calcium ion, is known to be involved in the regulation of many neurobiological processes, presumably by promoting synaptic vesicle-membrane interactions. Operating under the working hypothesis that CaM is the key agent which mediates many CaM-dependent processes, we investigated the role of CAMP in SCIP-1 expression. Northern analysis indicates that SCIP-1 mRNA is expressed at high levels in actively myelinating Schwann cells present in the peripheral and in cultured Schwann cells in a dose-dependent fashion. Kinetic analysis of this CAMP-mediated induction reveals that SCIP-1 is expressed only after the repression of the immediate early proto-oncogenes, c-jun, and precedes the expression of the myelin structural genes such as P0 and MBP. SCIP-1 (which stands for Schwann Cell Inducible EOU) may therefore represent an intermediate factor in the regulatory cascade of trans-acting factors that ultimately results in the formation of myelin in vivo.

503.4

SCIP-1: A CAMP-INDUCIBLE MEMBER OF THE POU TRANSCRIPTION FACTOR FAMILY EXPRESSED IN SCHWANN CELLS. E.S. Moon*, U.S. Weinmaster*, and G.E. Lemke, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

The factors involved in the regulation of myelin gene expression in the mammalian nervous system are currently unknown. In an attempt to isolate and characterize these factors, degenerate oligonucleotides were used to construct conserved regions of the POU family of cell-specific trans-acting proteins. Those oligonucleotides were used to screen a rat Schwann cell cDNA library, and a positive clone has been isolated and purified. Sequence analysis has revealed that this cDNA represents a novel POU factor that contains the conserved POU-specific cis-regulatory region characteristic of this family. Northern analysis suggests that this factor is expressed in a Schwann cell-specific manner. We have named the factor SCIP-1 (pronounced "skip").

Since in vitro studies suggest that CAMP is the key second messenger involved in regulating Schwann cell gene expression, we investigated the role of CAMP in SCIP-1 expression. Northern analysis indicates that SCIP-1 mRNA is expressed at high levels in actively myelinating Schwann cells present in the peripheral and in cultured Schwann cells in a dose-dependent fashion. Kinetic analysis of this CAMP-mediated induction reveals that SCIP-1 is expressed only after the repression of the immediate early proto-oncogene, c-jun, and precedes the expression of the myelin structural genes such as P0 and MBP. SCIP-1 (which stands for Schwann Cell Inducible EOU) may therefore represent an intermediate factor in the regulatory cascade of trans-acting factors that ultimately results in the formation of myelin in vivo.

503.5


An apparent selective loss of the magnocellular cholinergic neurons of the basal forebrain is observed as part of the pathology associated with Alzheimer disease (AD). A majority of the neocortically projecting magnocellular cholinergic neurons are located in the substantia innominata (SI). A cDNA library of genes from a normal SI was constructed and screened by a negative selection procedure to remove genes that are common to the cerebellum. The remaining clones were screened by differential hybridization with cDNA probes of normal and AD affected SI. From these, a 3 kb clone was isolated that recognizes a large transcript (15.5 kb). RNA hybridization analysis revealed that this gene is expressed in a number of brain regions (temporal and occipital cortex, hippocampus, caudate putamen, cerebellum and SI), but not in white matter, liver or placenta. Low stringency hybridization to RNA from peripheral organs. Homology between the human and rat genes appeared to be low. The DNA sequence obtained from this clone resembled to consensus sequences for binding of several transcription factors, including those for AP-2, the TATA factor, and the Box A factor. The simultaneous presence of the latter two sites is unusual, as the TATA sequence is commonly part of promoters utilizing RNA polymerase II (Pol II), while the Box A sequence is commonly used by Pol I. Our studies show that these elements may both independently direct proper initiation of human gfa transcription by Pol II, and that the sites likely interact synergistically.

503.6


Calmodulin (CaM), the principal eukaryotic intracellular receptor for calcium ion, is known to be involved in the regulation of many neurobiological functions controlled by calcium ion. For example, calmodulin has been shown to stimulate the phosphorylation of a number of proteins in synaptic vesicles, presumably by binding to and activating a synaptic Ca(II)-kinase. CaM has also been implicated in neurotransmitter release processes, presumably by promoting synaptic vesicle-membrane interactions. Operating under the working hypothesis that CaM is the key agent which mediates many CaM-dependent processes, we investigated the mechanism by which CaM physically interacts with its "targets: (both metal ions and enzymes) in the nerve cell, utilizing paramagnetic resonance (EPR) spectroscopy (see J. Inorg. Biochem. 33, 139-147, 1988). Part of this effort involves the production of bovine calmodulin variants, by employing the techniques of site-directed mutagenesis, which will facilitate the attachment of specific EPR probes to critical regions of the CaM molecule, such as at the proposed target enzyme binding site in the central helical region.
towards two-dimensional trypsin maps of 125I-labeled peptides of CHO-expressed CK-II. The role of phosphorylation on translocation of the 50 kDa detectable 50 kDa protein was present in non-transfected CHO cells. The first phosphoprotein was a 50 kDa polypeptide recognized by monoclonal antibodies and subsequently identified as CK-II. Additionally, two dimensional tryptic maps of 125I-labeled peptides of CHO-expressed 50 kDa protein and the 50 kDa subunit of rat purified CK-II showed the same pattern, confirming that the 50 kDa protein represented the subunit of rat brain CK-II. No immunodetactable or autocatalytically detectable 50 kDa or nontransfected CHO cells. The second phosphoprotein was approximately 58 kDa and migrated on two-dimensional isoelectric focusing /SDS-PAGE in a fashion similar to tubulin. Purification of the 50 kDa protein from CHO cells by CaM-Sepharose chromatography yielded a 67-70% pure kinase. Time dependent phosphorylation as well as the amount of CaM required to half maximal activating both enzymes were indistinguishable. Km values for synthesis phosphorylation were approximately 2-3 uM and Vmax values were 1.3-1.5 umol/min for both enzyme preparations. Like rat forebrain CK-II, after Ca2+-CaM-dependent autophosphorylation, the expressed 50 kDa subunit no longer required Ca2+/CaM for further auto- and substrate-phosphate mobilization. The role of phosphorylation on translocation of the 50 kDa subunit was also studied.

503.9

Astrocytomas, including glioblastoma multiforme, represent the most frequent and deadly neoplasms of the central nervous system. Despite numerous cytogenetic and oncogene studies, the primary mechanism of tumorgenesis in astrocytic tumors remains obscure. Recent evidence suggests that the loss or inactivation of certain "tumor suppressor" loci leads to the formation or a variety of human cancers. e.g., retinoblastoma and acoustic neuroma.

For this reason, fourteen astrocytomas of varying grades of malignancy were analyzed with a battery of polymorphic risk markers to search for specific chromosomal deletions. Although not the only genetic aberration seen, the most commonly detected losses were found for loci on the short arm of chromosome 17. This region does not include the gene causing Von Recklinghausen's neurofibromatosis (NF 1).

Interestingly, loss or inactivation of both copies of p53, a putative "tumor suppressor" gene located on chromosome 17, has recently been implicated in the tumorgenesis or colon carcinoma. The possible involvement of p53 as a candidate locus in astrocytoma development is currently studied. Characterization of this and other "tumor suppressors" loci may shed insight into fundamental mechanisms of tumorgenesis in the central nervous system.

503.11

L7 is a gene that encodes a protein shown to be exclusively expressed in cerebellum Purkinje cells (Oberdick, J. et al. 1986; 1988), but which we now show also to be expressed in a restricted population of retinal neurons, the bipolar cells. Seven unrelated markers isolated in our lab, namely PEp-19 (Dav. R. et al. PNAS 83, 8430, 1986) and cerebellin (Bammon, J. et al. PNAS 82,7145, 1985), share the L7 gene restriction within the cerebellum, but all three markers are not localized patterns throughout the remainder of the brain. L7 and cerebellin are the most restricted, while PEp-19 is more widely expressed. We believe one of the goals of our research is to delineate regions of control within the respective marker genes which 1) provide Purkinje cell specificity and 2) control their unique spatial and temporal patterns. One might expect the former to be reflected in shared elements within marker gene promoter sequences, the latter in differences. As a first step in this analysis we report the cloning and sequencing of L7 genomic DNA from rat and mouse, and the identification of non-coding regions which are highly conserved between the two species. Experiments that have used unique loci in trans are being undertaken and will be discussed. In addition we have found that L7 protein expression is controlled by translational processes that are differentially regulated during Purkinje cell development resulting in two forms of L7 in adults. Only one form of L7 appears in the retina. At least one mutation affecting the coding region of L7 is responsible for the disease.

Experiments designed to identify this tissue specific and temporally regulated modification process have been initiated. Partially supported by PHS Grant #1R01-004.

503.12
NEURONAL GROWTH RESPONSE GENES EXPRESSED IN CANARY SONG CONTROL REGION HVC: CLOSING OF EGR-1/NGFI-A HOMOLOGUES. C. Melle, M. Penaerts and B.P. Clayton. Lab. of Animal Behavior, The Rockefeller University, NY 10021

We are trying to identify and analyze genes which act as primary regulators of neuronal plasticity, and have been focusing on the higher vocal center (HVC) of canaries. HVC undergoes significant seasonal and steroid-regulated changes in volume in adulthood, reflecting changes both in neuronal growth and neuron number. Egr-1/NGFI-A is a candidate primary regulator gene, isolated in mammals by several investigators independently (Cell 53:37, Science 238:797, PNAS 85:4691). It is induced rapidly following stimuli for the "multiple zinc finger" structure believed to be characteristic of a class of transcriptional regulators. To look for expression of homologous genes in HVC, we screened an HVC cDNA library at low stringency with cDNA clones provided by Y. Shukatne (mouse Egr-1) and J. Milbrandt (rat NGFI-A). We have identified at least one clone with a high degree of sequence similarity in the HVC library, and are conducting further analyses. We speculate that the expression of an Egr-1/NGFI-A homologue in HVC may serve an important function in regulating other genes involved in the plasticity of this brain region.

The a subunit of the Na+, K+ATPase contains the catalytic site for ATP hydrolysis and the binding site for cardioactive glycosides. Three isoforms of the a subunit are encoded by three distinct genes. To analyze the transcriptional control of these genes, we have isolated cDNA clones of the genomic sequences for the mouse at, a2 (aII), and a3 (a+) genes, average 40 kb in length, in the vector pκE15. Restriction mapping and Southern blot analysis of the probes showed that two overlapping cDNAs which span the entire coding region of the aI gene contain a common 2.5 kb aI genomic sequence. The mouse aI gene is estimated to be 30 kb in length. Co-transfection of the two cDNAs into oocytes-sensitized CV-I monkey cells by calcium phosphate procedure confers oocyte resistance to these cells, with an average yield of 72 resistant colonies/106 cells/μg DNA. No oocyte-resistant colonies were observed with transfection of either aI alone. Subsequent analysis of the transfectants demonstrated that the two cDNAs have integrated into the CV-I cell chromosomes and undergone homologous recombination in the overlapping region to form a functional mouse αI gene. Functional and DNA sequence analyses of the transcriptional control elements for these three genes are currently in progress.

MOLECULAR CLONING OF A cDNA ENCODING A POSTSYNAPTIC DENSITY PURIFIED FROM THE MAMMALIAN BRAIN. In order to investigate the structure and function of the post-synaptic densities purified from the mammalian brain, we have isolated and sequenced a cDNA encoding a protein from these brains. The isolated cDNA encodes a protein that is highly homologous to the postsynaptic density protein. This homology suggests that these proteins may play a role in the formation and function of synapses. We are currently investigating the expression and function of this protein in various brain regions and in response to different stimuli.

IDENTIFICATION OF RETINA-SPECIFIC BINDING SITES IN THE RAT OPSIN PROMOTER. M.A. Morabito* and C.J. Barnstable. Yale University School of Medicine, Dept. of Ophthalmology and Visual Science, New Haven CT 06510.

The opsin gene is expressed postnatally in the rod photoreceptor cells and is developmentally regulated at the transcriptional level (Tretiak J.E. et al. Mol. Cell. Biol. 8:1567-79, 1988). Opin transcripts can first be detected at PN1 and the rate of transcription increases 30 fold to reach adult levels at PN10. A 6.5 Kb EcoRI fragment isolated from a rat genomic DNA library contains the complete coding sequence and the promoter region of the rat rod opsin gene. Like other mammalian opsin genes the structural portion consists of five exons interrupted at distinct sites in the introns. The coding portion of the gene is highly homologous to the corresponding regions of other mammalian rod opsin genes (86-90%) and these values are even higher when the amino acid sequences are compared. The homology in the promoter is limited to small regions of DNA like the TATA and CAAT boxes.

The 500 bp of the promoter region proximal to the transcriptional start site of the rat opsin gene contains four CAAT box regions identified by homology with the consensus sequence CAAT and two of these are flanked by short direct repeats. We have previously shown that the CAAT box located at position -121 interacts with a retina nuclear extract to form a unique DNA-protein complex not present when using extracts from other CNS regions. We have now identified two regions of the rat opsin promoter that bind specifically to the retina extract forming unique DNA-protein complexes not present using brain, cerebellum or retina extracts. Supported by NIH grants EYS02506 and NS02483.


Gap-43, an abundant growth cone membrane protein, is encoded by a single-copy gene whose transcription is strongly correlated with axon development and regeneration (Cell 49:785,1987). We have isolated coding and non-coding portions of the rat GAP-43 gene to investigate the organization of protein-coding structural domains, and DNA sequences that may contribute to the regulation of GAP-43 expression. Restriction mapping of rat genomic DNA, and sequence analysis of cloned genomic DNA, show that the GAP-43 gene contains three coding exons. The 5' exon (Exon 1) encodes a small (10 aa) amino-terminal domain containing the proposed site for fatty acyl and membrane binding of the protein. Exon 2 codes for the majority of GAP-43, including the proposed calmodulin-binding site and the carboxy-terminal domain with partial homology to neurofilament proteins. One interpretation of this organization is that GAP-43 comprises a core domain that interacts with intracellular binding proteins. We are studying the regulation of this gene in vitro by transfecting GAP-43 promoter-cat gene constructions into cultured Schneider cells and exposing them to various extracellular factors. Supported by grants SS2170 and SS01282-02. V.R. is the recipient of a CIDA from the NINDS.

MOLECULAR CLONING OF A cDNA ENCODING A POSTSYNAPTIC DENSITY GLYCOPROTEIN. B.NL. J.W. Gard and I.R. Brown. Department of Zoology and Biochemistry, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C1A4.

We have raised a polyclonal antibody to the post-synaptic density glycoprotein PS-D380/65/6 which is concentrated in densities purified from the mammalian brain. In order to investigate the structure and function of the PS-D380/65/6, we have used an aminoterminal fragment of this protein as a probe to test the potential to form the left-handed z-DNA conformation. Unusual topology resulting from the interactions of these two sequence elements may contribute to the regulation of GAP-43 expression. Supported by NIH grant EY07397.

MOLECULAR CLONING OF THE HUMAN MYELIN P2 PROTEIN GENE: ANALYSIS OF ITS STRUCTURE AND REGULATION. V. Narayanan and G. Pettenkofer. Dept. of Neurology, The Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Myelin P2 protein, a 15 kDa cytosolic basic protein, is synthesized in Schwann cells and oligodendroglia. It belongs to a family of fatty acid binding proteins, and thus may have an important metabolic role during myelination. Our goal is to study the expression of this protein, investigate the nature of extracellular signals that initiate and regulate the gene, and study the mechanisms of this genetic control. We had previously characterized a cDNA clone encoding rabbit myelin P2 protein (J. Biol. Chem. 263 8322 (1988)). We used this as a probe to screen a human genomic library constructed in λFIX (Stratagene Inc.) at low stringency. We identified two positive clones containing parts of the P2 gene. One of these, HLP14.2.1, was partially sequenced and found to be similar to the first exon of this gene. The splice site between this exon and the first intron occurs at an amino acid (Gly), identical to the situation for the mouse myelin P2 gene and others in this family of fatty acid binding proteins. We are studying the regulation of this gene in vitro by transflecting P2 promoter-cat gene constructions into cultured Schwann cells and exposing them to various extracellular factors. Supported by grants SS21700 and SS01282-02. V.R. is the recipient of a CIDA from the NINDS.


Utilizing the enzyme Blase the chromatin conformation of cortical neurons has been recently analyzed (Neurochem. Res. 14:129-137, 1989). In this study we demonstrate the presence of 4 tissue-specific Blase I hypersensitive sites flanking the mouse 68 kDa neurofilament gene. One of these sites maps to a region near the TATA box while the other 3 sites are further upstream. We have further characterized the coding sequence by NcoI/BamHI restriction analysis. A 1.8 kb NcoI/BamHI fragment recognized on immunoblots by AbI/BamHI and an ii) polyclonal antibodies raised against the fusion protein from AbI/BamHI digested fusion protein by NDRI/BamHI. A 739 bp cDNA insert from NcoI/BamHI detected two abundant mRNA species (1.8 and 3.8 kb) which are expressed at high levels in brain and at much lower levels in muscle. Interestingly, DNA sequencing data for this 350 bp cDNA reveals substantial similarity but not identity with the cDNA sequence for calmodulin. Pull down experiments have been obtained by hybridizing another brain cDNA library with the NcoI/BamHI insert. The restriction map of this cDNA is different from that known for calmodulin binding. (Supported by grants from NSERC to I.B. and MRC to J.G.)
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350.19
STRESS ACTIVATION OF C-FOS EXPRESSION IN RAT BRAIN. H. Alho, J. Kononen*, J. Kostialin* and A. Hervonen, Dept. of Biomedical Sciences and Dept. of Public Health, Univ. of Tampere, 33101 Tampere, Finland.

The proto-oncogene c-fos protein (Fos) is expressed in the nuclei of neurons in rodent brain. The basal expression of Fos is relatively low in most central nervous system regions and increases after generalized seizures. Whether the Fos can be included in response to synaptic activation by stress, rats were stimulated by immobilization and Fos expression was examined immunohistochemically.

Adult rats were immobilized for 1.2 and 3 h. Sections were stained for Fos staining 1 h after the end of immobilization. The protein antisera was raised to a synthetic M-peptide region. The stimulated rats exhibited increased Fos immunoreactivity. This was the most intense three hours after the immobilization. The major differences from control brains were the appearance of the Fos immunoreactivity in the paraventricular, habenular, arcuate, accumbens and septal nuclei. In cortex, the intensity of staining was increased in stimulated animals. The induction of Fos in adult neurons suggests that this protein may have role in CNS related to synaptic activation by stress.

350.21
LIGAND AUTORADIOGRAPHIC RECEPTOR SCREENING: VALIDATION USING BETA ADRENERGIC RECEPTOR cDNA. M. Battaglia, S.L. Lauter, and D.R. Uhlig (SPON: J.L. Rutkowski) NIDA/Adiction Research Center, Baltimore MD 21224.

In order to validate a ligand-autoradiographic receptor screening (LARS) methodology, we have used hamster beta adrenergic receptor (BAR) cDNA to express a beta-adrenergic receptor (BAR) cDNA expression vector (pBAR), a gift of Dr. R. Dixon. This plasmid contains sequences which allow replication and cDNA expression in permissive SV40 T-antigen expressing cell lines (i.e.: COS, but not CV1 cells) We expressed foreign DNA in CV1 or COS cells, produced replicas of the transfected cells on polyester filters and identified BAR expression by 125I-CYP receptor autoradiography. When pBAR was introduced into cells by speroplast fusion, COS cell colonies with high levels of beta-receptor binding were observed on filter autoradiographs. None were found on filter replicas of CV1 cells. Binding was displaced appropriately by propranolol and isoproterenol. When pBAR-containing speroplasts were mixed, in decreasing proportions, with appropriately by propranolol and isoproterenol, we were able to detect receptor autoradiography. Therefore, we have set out to identify proteins specific to the LC, and provide important information concerning the molecular basis of some of the unique functional properties of LC neurons.

350.23
LIPOFUSCIN: A HIGHLY EFFICIENT PROCEDURE FOR THE TRANSIENT AND STABLE TRANSFECTION OF NAIVE AND NGF INDUCED PC12 CELLS. Susanne Z. Moller* and Stuart C. Emeson (SPON: S. Fisher) Neuroscience Research Institute and Dept of Biological Sciences, Univ of Calif, Santa Barbara, CA 93106.

Introduction of in vitro manipulated DNA into cultured cells is one of the most powerful methods for dissecting gene function. Unfortunately, a subset of cells lines transfect at very low efficiencies, thereby precluding many analyses. The NGF responsive cell line PC12, among the most widely studied cell lines in neurobiology, has been among these low efficiency lines. We have overcome this experimental impasse by adapting the lipofection procedure (Felgner et al. NASE 88:7413). We have found that liposomes containing DNA transfect cells at extremely high efficiencies. Efficient transient transfection of PC12 cells, which has not been possible by other procedures, has been demonstrated by transfection of the plasmid pSV-CAT. This vector uses a Rous Sarcoma VirusLTR to direct transcription of a chloramphenicol acetyl transferase (CAT) reporter gene. Additionally, we have demonstrated that lipofectin can transfect NGF differentiated PC12 cells, allowing the transient introduction of genes into PC12 cells at any stage of their differentiation. Stable transfection, as assayed by loss of puro 614 (confined by integration of the neo gene), occurs at a frequency of 800 transfecants per 10^6 cells. This is 100 fold higher than obtained using the standard CaPO_4 precipitation (Schweitzer et al. J. Cell Biol. 101:667). The availability of this technology will greatly increase the range of experimental manipulations possible in studying the mechanisms of NGF action and other issues in PC12 cells. (Supported by the Muscular Dystrophy Association and NIH Grant #RC21-N24367)

350.20

Previous evidence suggests that expression of some proto-oncogenes in adult neurons may be activity-dependent. 45 minutes after the onset of seizuers produced by i.p. injection of 300mg/kg pilocarpine (detected by immunohistochemistry) is increased substantially in neurons of the hippocampal dentate gyrus; uptake of [3H]-deoxyglucose (20%; detected by autoradiography) is also greatly increased within the dentate gyrus. Prior treatment with scopolamine (1mg/kg, s.c.) blocks these effects. 3 hours after the onset of tremor produced by i.p. injection of 15mg/kg harmaline, c-fos protein (absent normally) is detected in neurons of the inferior olivary nucleus, and uptake of 2DG is greatly increased within the same region. After pilocarpine, c-fos induction and increased 2DG uptake are not detected in the substantia nigra, a possible site of seizure initiation, but occur in the secondary affected dentate gyrus. After harmaline, c-fos induction and increased 2DG uptake occur in the inferior olive, the site of tremor initiation of seizures. We have used molecularly defined c-fos inducer to compare and contrast c-fos protein expression and [3H]-deoxyglucose uptake in brain regions.

350.22
IDENTIFICATION OF LOCUS COERULEUS-SPECIFIC PROTEINS BY PROTEIN PHOSPHORYLATION AND SUBTRACTION HYBRIDIZATION. K. Saijo*, R.S. Duman, and E.J. Nestler. Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Locus Coeruleus (LC), the major noradrenergic nucleus in brain, exhibit many distinct characteristics based on anatomical, electrophysiological, and pharmacological studies. Therefore, we have set out to identify proteins specific to the LC, and have identified a number of mRNA transcripts enriched in this brain region. In one series of experiments, we have used protein phosphorylation and two-dimensional electrophoresis to detect transcriptional changes in specific phosphoproteins. To date, we have identified 4 phosphoproteins that appear to be enriched in the LC, in that they are either not detectable, or present at much lower levels, in a large number of other brain regions studied. Of particular interest is an acidic, 62 kD protein, which is regulated in the LC by chronic in vivo morphine treatment.

In a second series of experiments, we have used subtractive hybridization to identify proteins specifically expressed in LC neurons. We have constructed a cDNA library of LC and of other brain regions using this mRNA, and will carry out subtraction hybridization to isolate and then clone LC-specific messages. Identification of proteins expressed uniquely in the LC will provide important information concerning the molecular basis of some of the unique functional properties of LC neurons.

The deoxymethyl code (DOC-salt model) of hypertension has been shown to be VP-dependent. In these studies, we investigated whether levels of VP mRNA in the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) change during the development of DOC-salt hypertension. Male Sprague-Dawley rats (80-90 gm) were unilateral nephrectomized (day -14) and 7 days later (day 0) their drinking water was replaced with 0.9% saline. A week later, RCA capsules containing DOC (100 mg/kg in sesame oil) or saline (the sham group) were implanted in the bladder and 30 and 24 hrs later, DOC and saline were withdrawn. Mean arterial pressure (MAP) was measured, animals were sacrificed and tissue samples of the magnocellular nuclei and the son of the paraventricular nucleus were removed. A semiquantitative autoradiography was used to measure VP mRNA in SON, PVN and SON. Immunocytochemistry was performed to quantify VP immunoreactivity. A decrease in VP mRNA levels in the SON (sham: 77.5 ± 6.5 cells; DOC: 67.8 ± 12.0 cells) and a significant increase in VP mRNA levels in the PVN (sham: 9.0 ± 4.0 cells; DOC: 19.0 ± 4.0 cells) was observed in DOC-salt hypertensive rats compared to sham-control. Thus, VP neurons in PVN, possibly a subpopulation which projects to cardiovascular regulatory centers, may be important in the onset of DOC-salt hypertension.


Expression of vasopressin (VP) mRNA in the paraventricular nucleus is influenced by glucocorticoids. In order to determine whether degeneration of the cells in the bed nucleus of the stria terminalis (BNST) and medial amygdala (AM) respond to changes in glucocorticoid levels, in situ hybridization and quantitative autoradiography with [35S]ATP were used to measure VP mRNA in the BNST and AM of rats that were sham-operated (SH), adrenalectomized (ADX: 14d) and dexamethasone treated. Results show a significant increase in the number of labeled cells in the BNST (SH: 75±4; ADX: 64±3; AME: 76±8, 14d) and later sectioned (12u) on a cryostat. In situ hybridization was performed on brain sections using an oligodeoxynucleotide probe complementary to VP mRNA (Lightman & Young. J. Physiol. 394:21, 1987) and labeled with [35S]ATP. Following hybridization, sections were placed against autoradiographic film. The relative density and area of each nucleus was quantified using a Bioquant IV Image Analysis System. DOC treatment decreased MAP (days 5,12,30), urinary VP output (days 5,12,30) and plasma VP (day 30). VP mRNA levels (density) in the PVN were elevated 8.6% and 16.5% on days 12 and 30, respectively, in DOC-treated rats compared to sham. No significant changes in VP mRNA were observed in SON. Thus, VP neurons in PVN, possibly a subpopulation which projects to cardiovascular regulatory centers, may be important in the onset of DOC-salt hypertension.
504.7


Our previous studies have shown that in the neostriatum, the expression of preproenkephalin (ppE) mRNA is regulated by glucocorticoids. There is a decrease in striatal ppE mRNA after adenectomy (ADX) and ADX animals replaced with corticosterone express higher levels of striatal ppE mRNA than ADX animals. In this study we have used in situ hybridization to assess the level of ppE mRNA expression. We determined that the increase in striatal ppE mRNA is evident after 16 hours, but not after 2 hours, of corticosterone replacement in ADX animals. The effect of corticosterone treatment on ppE mRNA expression in ADX rats is not mimicked by the increase in endogenous corticosterone produced in situ stress and as we have shown previously, the increase in endogenous corticosterone produced as a result of stress may affect the expression of genes of interest. We are currently investigating the effects of chronic stress and of the diurnal variation in endogenous glucocorticoid levels on ppE mRNA expression. (Supported by MH41256 and NS07080.)

504.8


Dept of Physiol. & Biophys. and Ob. & Gyn., U. of Wash., Seattle, WA 98195.

Sexually differentiated secretory patterns of pituitary hormones are thought to be orchestrated by a steroid-sensitive neural network, which becomes organized during a critical period in neonatal life. Sex differences, derived from the prohormone molecule POMC, has been implicated in the regulation of anterior pituitary function. We sought evidence for sexual dimorphism in POMC gene expression in cells of the arcuate nucleus by comparing POMC mRNA content within individual neurons between male (n = 3) and prococious female (n = 3) rats. Animals sacrificed and brain slices through the arcuate were prepared for in situ hybridization with an RNA probe for the POMC message. Neurons were identified with a computerized image processing system. Grains/cell used as an index of POMC mRNA content.

504.9

EXPRESSION OF HYPOTHALAMIC NEUROPEPTIDES IN FOOD-RESTRICTED RATS. L.S. Brady, M.A. Smith, P.W. Gold and M. Herkenham. Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Disturbances in CNR peptide systems are evident in patients with eating disorders such as anorexia nervosa. We examined mRNA expression of hypothalamic peptides in food-restricted rats to characterize the consequences of weight loss. Food intake was restricted (10%) in male and female Sprague-Dawley rats for two weeks; animals lost 30% of their body weights. Crystall-cut sections through the hypothalami were hybridized with 35S-oligonucleotide probes for pro-opiomelanocortin (POMC), neuropeptide Y (NPY), corticotropin releasing hormone (CRH) and other peptides. Sections were exposed to film and autoradiograms were then dipped in nuclear emulsion. POMC message was reduced in the arcuate nucleus by 43-47% relative to the control group; this change represents a 26-36% decrease in the number of labeled cells and a 59-66% decrease in grains/cell. NPY message was increased in the arcuate nucleus by 30-58%; the number of labeled cells increased 70-155% and grains/cell was increased 117-150%. CRH, NPY, dynorphin and vasopressin mRNAs were not altered in the paraventricular nucleus. These results suggest that decreased CRH and increased NPY concentrations in the CSF of anorexia patients reflect normal physiological consequences of weight loss. Increased secretion of CRH and other peptides in the CSF of anorexics may reflect pathological consequences of the disease.

504.10


Previous work from this laboratory has demonstrated the expression of preproenkephalin (PPE) mRNA in cultured astrocytes. PPE mRNA was found in both embryonic and neonatal astrocytes isolated from different brain areas, with peaks at postnatal days 1, 4, 7, 10, 14, 29 and 25 days old, respectively. Northern blots containing total cellular RNA were hybridized with a riboprobe for preproenkephalin mRNA. PPE mRNA levels in treated cultures increased 3-5 fold over controls by 16 hr. The isopropenyl-stimulated increase could be blocked almost entirely by treatment with the β-antagonist propranolol. This increase could be mimicked by treatment with 5 µM forskolin. These data strongly suggest that astroglial PPE mRNA can be stimulated by a cAMP transduction system, and that this system can be activated through β-adrenergic receptors. Levels of PPE mRNA were also stimulated by treatment of cultured astrocytes with 100 µM cortisone-arabinoide, raising the possibility that PPE mRNA metabolism may be related to the cell cycle. In vivo, these observations suggest that levels of preproenkephalin mRNA can be regulated by several different physiological stimuli.

504.11


Corticotropin-Releasing Hormone (CRH), is a neuropeptide involved in hormonal and behavioral stress responses in mammals. The adrenal axis and the stress response to CRH are depressed in the peri-natal period, possibly protecting the brain from high, toxic steroid levels generated by the stress of birth. The role of CRH in the stress response and the peptide's expression in hypothalamic and extra-hypothalamic areas during critical periods have not been elucidated. We have investigated the expression of CRH gene in rat brain, and especially in the paraventricular nucleus (PVN) by in situ hybridization using an S35 labeled synthetic 60-mer oligonucleotide probe, with a sense strand control. Message is first detectable on the 18th fetal day in the PVN, which is compatible with the formation of the paraventricular cells on the 17th fetal day (Altman and Bayer, 8A, Adv. Anat. Emb. Cell Biol. 100, 1-78, 1986). Probe signal is also increased on the 18th fetal day in the ventral septal region. Message decreases perinatally in both regions, concurrent with the non-responsive period of the CRH-adrenal axis, suggesting that a decrease in CRH gene expression may contribute to this phenomenon.

504.12


Corticotropin-Releasing Hormone (CRH), is a 41-residue peptide hormone that is widely distributed in the brain. CRH is synthesized as a prohormone (POMC) and released by stimulation of the hypothalamic CRH neurons. CRH is released by hypothalamic neurons that project to the pituitary to stimulate ACTH release and to extrahypothalamic regions to regulate a variety of behaviors and neural functions. POMC is a prohormone containing 3 peptides, ACTH, ß-endorphin, and MSH. CRH is synthesized as a precursor protein consisting of 103 amino acids, and it is cleaved from the POMC precursor by a specific prohormone-converting enzyme (PCE). CRH is a potent stimulator of ACTH secretion and has a number of other effects, including vasopressin and dopamine release, as well as an inhibitory effect on pituitary secretion of growth hormone. CRH has been shown to modulate a variety of physiological functions, including the immune system, the endocrine system, and the nervous system. CRH has been shown to modulate the immune system, including the release of cytokines and other immune mediators, and has been implicated in the regulation of the immune response. CRH has also been shown to modulate the endocrine system, including the release of ACTH and cortisol, and has been implicated in the regulation of the stress response. CRH has also been shown to modulate the nervous system, including the release of dopamine and other neurotransmitters, and has been implicated in the regulation of mood and behavior. CRH has been shown to modulate the immune system, including the release of cytokines and other immune mediators, and has been implicated in the regulation of the immune response. CRH has also been shown to modulate the endocrine system, including the release of ACTH and cortisol, and has been implicated in the regulation of the stress response. CRH has also been shown to modulate the nervous system, including the release of dopamine and other neurotransmitters, and has been implicated in the regulation of mood and behavior. CRH has been shown to modulate the immune system, including the release of cytokines and other immune mediators, and has been implicated in the regulation of the immune response. CRH has also been shown to modulate the endocrine system, including the release of ACTH and cortisol, and has been implicated in the regulation of the stress response. CRH has also been shown to modulate the nervous system, including the release of dopamine and other neurotransmitters, and has been implicated in the regulation of mood and behavior.
104.13

EFFECTS OF CHRONIC ANTI-PSYCHOTIC DRUGS ON PREPROTACHYKININ mRNA IN RAT FOREBRAIN. J. Shibata, D.M. Haverstick, and J.G. Sutcliffe. (SPON: University of Illinois at Chicago)

While tachykinin mRNA detection in the rat brain has been intensively studied, very little is known about the regulation of tachykinin gene expression in limbic areas. We have used a 35S-labeled antisense riboprobe to detect the in situ hybridization of prepro-tachykinin mRNA in various rat forebrain areas. These areas include the hypothalamus, amygdala, striatum, and midbrain. We have found significant differences in the levels of prepro-tachykinin mRNA in these various regions. In addition, we have detected an increase in the level of prepro-tachykinin mRNA following chronic administration of haloperidol. This increase is seen in both the hypothalamus and amygdala, but not in the striatum and midbrain. We have also detected a decrease in the level of prepro-tachykinin mRNA following chronic administration of lithium. This decrease is seen in both the hypothalamus and amygdala, but not in the striatum and midbrain. We have also detected a decrease in the level of prepro-tachykinin mRNA following chronic administration of lithium. This decrease is seen in both the hypothalamus and amygdala, but not in the striatum and midbrain.

104.14

DEVELOPMENTAL PROFILE OF PREPROTACHYKININ mRNA IN RAT STRIATUM. D.M. Haverstick, D. Niesiotis, and J.G. Sutcliffe. (SPON: St. Louis University)

The developmental profile of prepro-tachykinin mRNA in the rat striatum has been studied using in situ hybridization. In the rat striatum, prepro-tachykinin mRNA is first detectable at postnatal day 10, and reaches its peak at postnatal day 15. The decline in expression occurs rapidly after postnatal day 15, with a significant reduction in mRNA levels by postnatal day 30. The decline in expression is gradual and continues until postnatal day 60, at which point the level of mRNA in the striatum is less than 50% of the peak level observed at postnatal day 15. The decrease in expression is seen in both the dorsal and ventral striatum, but is more pronounced in the ventral striatum. The decrease in expression is not due to a decrease in the number of neurons expressing prepro-tachykinin mRNA, as determined by in situ hybridization and immunohistochemistry. The decrease in expression is due to a decrease in the amount of prepro-tachykinin mRNA in the cells expressing the gene.

104.15


We have used in situ hybridization with a 35S-labeled antisense riboprobe to detect the in situ hybridization of neurotensin/mneromedin (NTN) mRNA in various rat forebrain areas. Our results indicate that the highest levels of NTN mRNA are found in the hippocampus and subiculum, with lower levels in the neocortex, amygdala, hypothalamus, and thalamus. The levels of NTN mRNA in the hippocampus and subiculum are similar to those found in the neocortex, but are much higher than those found in the amygdala, hypothalamus, and thalamus. The levels of NTN mRNA in the hippocampus and subiculum are highest in the dentate gyrus and molecular layer of the hippocampus, and in the subiculum, respectively. The levels of NTN mRNA in the neocortex are highest in the prefrontal cortex, and in the piriform and retrosplenial cortex.

104.16

THE ACUTE EFFECTS OF HALOPERIDOL ON NEUROTENSIN mRNA IN RAT STRIATUM AS DETERMINED BY FLUORESCENCE IN SITU HYBRIDIZATION. F.G. Williams*, M.P. Murtaugh*, A.J. Beitz* (SPON: University of Michigan, Ann Arbor, MI 48109).

The effects of haloperidol (HAL) on the expression of neurotensin (NT) mRNA in the rat striatum were studied using in situ hybridization. In the striatum, HAL caused a significant increase in the number and distribution of NT-immunoreactive cells. The increase in NT-immunoreactive cells was seen in both the dorsal and ventral striatum, and was most pronounced in the ventral striatum. The increase in NT-immunoreactive cells was accompanied by an increase in the level of NT mRNA, as determined by in situ hybridization and immunohistochemistry. The increase in NT mRNA was seen in both the dorsal and ventral striatum, and was most pronounced in the ventral striatum. The increase in NT mRNA was accompanied by an increase in the level of NT immunoreactivity. The increase in NT mRNA and immunoreactivity was seen in both the dorsal and ventral striatum, and was most pronounced in the ventral striatum. The increase in NT mRNA and immunoreactivity was accompanied by an increase in the level of NT release.

104.17


The rat 1807Y mRNA encodes a 533-residue novel, secretorain-like acidic protein with an apparent signal secretion, several pairs of tandem basic residues, and internally repeated sequence elements. 1807Y transcripts are detected by blotting and in situ hybridization at high levels in frontal and striatal cortex, the bed nucleus of the stria terminalis and piruitary corticothor, at lower levels in most other regions, but not in several other tissues. Utilizing antisera to synthetic peptide fragments of the predicted protein sequence, and to a pituitary-specific 57 kDa acidic protein that in immuno- histochemistry experiments is found in cellular processes and fiber tracts, generally consistent with axonal transport from the cell bodies identified by in situ hybridization. Although the function of the 1807Y protein is presently unknown, a mouse ablated for the 1807Y- homologous gene has been produced, that allows further studies of the potential involvement of this molecule in the secretory pathway.

104.18


Neuropeptide Y (NPY) potently stimulates food intake in rats by acting within the hypothalamic paraventricular nucleus and the content of NPY within the paraventricular nucleus has been shown to respond to food deprivation and refeeding. In this study we examined the possibility that NPY gene expression is increased following food deprivation by measuring the hypothalamic content of preproNPY mRNA. Adult male Sprague-Dawley rats were allowed free access to water and food, or were food restricted for 72 hours. At the end of the 72 hour period, the rats were killed and the hypothalamic content of preproNPY mRNA was determined. The results showed a significant increase in the hypothalamic content of preproNPY mRNA in rats that were food restricted for 72 hours, compared to rats that were allowed free access to food. The increase in preproNPY mRNA content was associated with a decrease in body weight, and was not due to a decrease in the number of neurons expressing preproNPY mRNA. The increase in preproNPY mRNA content was not due to a change in the number of preproNPY mRNA positive cells, as determined by in situ hybridization and immunohistochemistry. The increase in preproNPY mRNA content was due to a change in the level of preproNPY mRNA in the cells expressing the gene. This suggests that the increase in preproNPY mRNA content is due to a change in the level of preproNPY mRNA in the cells expressing the gene.
PREPRONEUROPEPTIDE Y mRNA CONTENT IS SPECIFICALLY INCREASED IN HYPOTHALAMUS OF OBESE ZUCKER FATTY RAT
G. Samorajski, Komba Tlaia, J.A.D. Marti, M. Bernardini, D. Endocrinology, Dept. Medicine, SUNY, Stony Brook, NY 11794.
Neuropeptide Y (NPY) is a 36 amino acid peptide that potently stimulates catecholamine secretion, increases the hypothalamus of paraventricular nucleus in rats and when given chronically, can lead to hyperphagia and obesity. NPY immunoreactive cell bodies have been localized to the hypothalamus and project to the paraventricular nucleus. In this study, we investigated the possibility that preproNPY mRNA levels are increased in hypothalamic paraventricular nucleus in rats. PreproNPY mRNA levels were measured in eight additional brain regions (cortex, olfactory bulb, cerebellum, hippocampus, brain stem, striatum, thalamus and lateral geniculata). Preliminary results suggest that the increase in preproNPY mRNA levels is specific to the hypothalasus. In situ hybridization analysis confirmed that the increase in hypothalamic preproNPY mRNA content was localized to the arcuate nucleus. These data are consistent with the hypothesis that regulation of hypothalamic NPY expression is disturbed in obese Zucker rats and that this disturbance may play a role in the etiology of obesity in these animals. (NIH MH 42074 & SUNY Faculty Development Award).

DIRECT UPTAKE AND RELEASE OF [3H-N-ACETYLCYLSTEAMINE]GLUTAMATE FROM CHICK RETINAL NEURONS.
Data on the neuronal localization and synaptic release of glutamate (Glu) has stimulated interest in its extracellular fate, particularly in retinal tissue where we have demonstrated local release of Glu.
Chick retinas were incubated in vitro with [3H]-NAAG to assay both extracellular peptide activity against it and direct transport of the peptide into retinal cells. The transport of [3H]-NAAG was distinguished from transport of peptide-released [3H]-glutamate by incubation of retinal cell monolayers with inhibitors. Time-dependent [3H]-NAAG uptake into retinal cells was observed. The intracellular appearance of [3H]-NAAG was inhibited by extracellular peptide activity against [3H]-NAAG, a likely result of greater availability of Glu transporters. Human retinal cells were incubated with a series of physiological buffers to stimulate release after a 30 min incubation with [3H]-NAAG. [3H]-NAAG incorporated into retinal cells by direct uptake was released upon depolarization and this release process required extracellular calcium.

Cerebellar taurine biosynthesis occurs in two steps; cysteine sulfonate decarboxylase (CSD), using as the immunogen an ovine CSD-enriched fraction but none with a brain crude extract. In the cerebellum, numerous immunolabeled cells were found in the white matter and Purkinje cell layer labeled like astrocytes. Purkinje cells are the only labeled cell type in the nerve endings. In the molecular layer no immunolabeled cells were found. In the cerebellum, the distribution and number of labeled hippocampal neurons appeared normal with an increase in the density of hybridization associated with individual neurons. At later times, hybridization over dentate granule cells (10 hr post-HL) and CA1 pyramidal cells (17 hr post-HL) became evident such that by 24 hr post-HL, hybridization was elevated far above normal over stratum granulosum, CA1 stratum pyramidale and superficial entorhinal cortex. By 2 days post-HL, the granule cells were no longer labeled whereas hybridization remained elevated in CA1 stratum pyramidale and entorhinal cortex. By 4 days post-HL, hybridization appeared normal in all fields. These data demonstrate that seizures induce a transient increase in hippocampus expression in several populations of hippocampal neurons including cells not previously considered to contain this neuropeptide. (MH 42074 [JWD] & NS52678 [CMG]).

EVIDENCE FOR 3H-ORG 2766 [A SYNTHETIC ACTH FRAGMENT] UPTAKE IN RAT HIPPOCAMPAL SYNAPTOSOMES.
The presence of specific receptor/ligand sites for ACTH and its fragments have not yet been established. We have therefore, tested for the presence of uptake sites for 3H-Or 2766 on hippocampal synaptosomal preparations. Tissues were obtained from young Sprague-Dawley female rats (250 gm), homogenized and centrifuged at 10,000 rpm. The superantigranular preparation was incubated with or without excess unlabelled Org 2766 (35M to 10M) with or without excess unlabelled Org 2766 to saturate specific uptake sites. No binding was observed at cell body preparations that were incubated for 20 minutes at 37°C. The tissue was harvested on Whatman GF filter paper. The filters were placed in 5 ml of scintillation fluid and counted. Our results showed a saturable low affinity uptake site for 3H-Or 2766 into hippocampal synaptosomal preparations after a 20 minute incubation. Studies are under way to identify the 3H-Or 2766 uptake into serotoni-ergic neurons grown in vitro. This study is supported by Organon International and NSF BNS 8812892.
505.6

ROLE OF ARACHIDONIC ACID (AA) METABOLITES IN THE RELEASE OF VasoACTIVE INTESTINAL PEPTIDE (VIP) IN MOUSE CEREBRAL VESSEL ENDOTHELIAL CELLS. John A. Magnussen. Instut de Physiologie, Université de Lausanne, 1055 Lausanne - Switzerland.

In rodent cerebral VIP is contained in a homogenous population of radially-oriented smooth muscle cells in the neomurine cerebral arterial (AA), a K+ channel blocker, promotes a concentration- and Ca2+-dependent release of VIP from mouse cerebral cortical slices with a significant effect already at 50 µM. Over 70% of VIP release was blocked by 4-AP (4APVPR) and 70% of VIP release was blocked by 2 µM indomethacin (TTX). Mepacrine, an inhibitor of phospholipase A2 (PLA2) activity and hence of AA formation, inhibits 4APVR (IC50: 15 µM). Melatonin (0.1-10 µM) a PLA2 activator, activates AA release in a lipoprotein pathway by NGDA, ETYA and caffeic acid results in a concentration-dependent inhibition of 4APVR. Thus, the formation of AA metabolites of inositol play a role in the release of a peptide in the mammalian CNS. Furthermore, these observations, together with the previously reported potentiation of prostanoids of VIP-stimulated ACTH formation in mouse cerebral cortex (Schad, N., Schoenfarb, M. and Magistretti, P. Nature 328:637, 1987), indicate that AA metabolites may act at both the presynaptic (lipoprotein metabolites) and postsynaptic (cycloxygenase metabolites) levels to increase the "throughput" or "strength" of VIP-containing cortical circuits.

A Ca2+-dependent K+-evoked VIP release (KVR) was also observed; KVR is not inhibited by nifedipine (10 µM), ω-conoxin (1 µM) or Ca2+ (100 µM), while N-type (1 µM) decreases KVR by over 60%. This pharmacological profile discards the involvement of L- and N-type Ca2+ channels in VIP release, in contrast to the release of other neuropeptides which appears to be mediated by channels of the N-type.

505.6

THE EFFECT OF ADENOSINE ON TACHYKININ RELEASE FROM PERFUSED ENTERIC NERVE VARIETIES. R.M. Broad, T.J. McDonald and I.A. Cook. Dept. of Pharmacology and Toxicology and Medicine, Univ. of Western Ontario, London, Ont. N6A 5C1.

The release of tachykinins (TKs) from isolated enteric nerve varieties may allow useful modelling of neural regulatory mechanisms. We have used perfused enteric nerve varieties, prepared from the myenteric plexus of the guinea-pig ileum, to examine the release of TKs under flow conditions. Synaptosomes were prepared in 0.3 mm Ca2+ solution at 37°C and specific RIA's for Substance P (SP) and Neurokinin A (NK-A) were performed. The effects of adenine on TK release were measured and compared with the effects of KCl and other stimuli. Calcium-free solution (0.3 mm Ca2+) reduced both TK release and TK levels in the perfusate.

505.7


Astrocytes cultured from striatum, cortex, hippocampus, and hypothalamic neurons. Both astrocytes and neurons expressed the same specific types of astrocytes expressing the mRNA encoding these neuropeptide processing enzymes.
A fused silica tube 1 meter long and 20 μm inside diameter was filled with sample. The system was run at 30 KV during 30 minutes. The detection level of this system improved by the addition of a chopper and a locking amplifier. With the mixture of 5 amino acids (serine, valine, leucine, isoleucine and treomine) or 5 neuropeptides (neurotensin, metenkephalin, angiotensin and sulfated and unsulfated) the system was used as a fluorescence detection method for capillary zone electrophoresis.

**Table 1**

<table>
<thead>
<tr>
<th>Diet</th>
<th>CHP-LI, μg/dl</th>
<th>Creatinine, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>5.8 ± 1.7</td>
<td>14.2 ± 0.5</td>
</tr>
<tr>
<td>Protein</td>
<td>9.8 ± 1.9</td>
<td>15.6 ± 0.9</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Fluorescence Microscopy**

Fluorescence microscopy was used to improve the sensitivity detection level of primary amino compounds separated by capillary zone electrophoresis. A capillary carrier was built and set on the plate of a fluorescence microscope. A fixed silica tube 1 meter long and 20 μm inside diameter was filled with 0.05M sodium tetraethenyl buffer at pH 8.3 and inserted into the capillary carrier. An uncoated section of the capillary was covered and focused. A mixture of 5 amino acids (serine, valine, leucine and tryptamine) and 5 neuropeptides (neurotensin, metenkephalin, angiotensin and sulfated cholecystokinin-8) were derivatized with fluorescamine and scanned on a fluorescence imaging microarray system that was presumably due to the presence of proteases. The fact that this method was clearly characterized by the addition of the detection level of the fluorescence detection method for capillary zone electrophoresis.

**Neurotensin Complexes**

We have previously characterized that neurotensin (NT) complexes with dopamine (DA) with a stoichiometry of 1:1 and KP2 = 3.9 x 10^6. NT is a peptide with unique properties in that it appears to activate DA neurons at the level of the cell bodies but blocks the behavioral effects of DA release in the accumbens, DA axonal terminal field. Although NT possesses some neuroleptic-like properties, it does not displace the in vivo DA agonist DAGO (4) or sulpiride (3). NT and KP2 occur with a stoichiometry of 1:1 (KP2 = 1.3 x 10^8). Utilizing molecular modeling software designed to predict the conformations of peptides in aqueous solution, we found that the structure of KP2 can form a highly basic pocket containing Pro-Arg-Arg-Pro-Arg.

**Neurotensin Complexes with Dopamine and N-Propylapomorphine**

D.K. Adachi*, P.W. Kalivas and J.O. Schenk (SPON: D.K. Adachi). Laboratory of Behavioral Physiology, Medical School, Department of Physics, University of Los Andes, Venezuela

Overview of NT and FNP complexes at pH 10.6, heat treated, and purified as described by Hartig et al. (Arch. Biochem. Biophys. 267:448-458, 1988). CPI fractions were collected from alkylated papain Reacti-gel with 2 M sodium thiocyanate incorporated into 32-[35S]GTP*. CPI fractions were differentiated from low Mf cystatins and high Mf kininogens by immunoblotting using specific antisera such as MAb Y13-259. Results will be compared to ras G-proteins purified by conventional chromatographic procedures. Availability of affinity-purified ras P21-CPI's will facilitate studies on their roles in CNS protein turnover.

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Supported in part by Grant NIMH-16178.
INTERACTIONS OF ACETYLCHOLINESTERASE (ACHE) WITH PROTEASE INHIBITORS. H. S. Emminger* (SPON J. Chan). Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53706.

The alleged proteolytic nature of ACHE (see Chubb, 1904, in Cholinesterases - Fundamental and Applied Aspects, pp. 345-359) was investigated using the enzyme for the prototype inhibitors benzamidine, pepstatin A, 1-LE-2-macroglubin, soybean trypsin inhibitor, and lima bean trypsin inhibitor coupled to biotinyl Sepharose. Bovine serum ACHE binds to all the inhibitors tested except 1-LE-2-macroglubin. Three to ten times more ACHE binds to columns of benzamidine and pepstatin A than to columns of soybean and lima bean trypsin inhibitors. Studies using only benzamidine and pepstatin A shows that binding is not a universal property of ACHE, since differences of ACHE from mouse serum, brain and skeletal muscle bind to the inhibitors to varying degrees and not at all. ACHE binding to the protease inhibitor beads is reduced by benzamidine, while the binding in the presence of pepstatin A and other protease inhibitors is the same as control levels or is increased. Inhibitors of ACHE activity (edrophonium, BW283C51, atropine, curare and gallamine) reduce the amount of enzyme binding to benzamidine and pepstatin A beads. The results suggest that some ACHE may possess sites to which inhibitors of serine and aprotinin will bind and that this interaction is influenced by inhibitors of ACHE activity. These results are consistent with the notion that ACHE is an enzyme with at least two distinct active sites.

BOVINE PINEAL THREONINE-RICH DECAPEPTIDE INCREASES ANTERIOR PITUITARY DOPAMINE IN MALE MICE. B. Benson, Department of Anatomy, University of Arizona, Tucson, AZ 85724.

During the course of large scale purification of bovine pineal peptides with mouse mammary milk-ejection activity (ME-activity), a peptide was identified which increased anterior pituitary dopamine (DA) in mice. Purification from more than 5 kg of defatted pineals was accomplished by homogenization in 0.2 M acetic acid, centrifugation (10,000 X g/20 min) at 4°C, Amicon ultrafiltration and Sephadex G-25 gel filtration. Subsequent isolation was achieved by serial semipurification HPLC on C-8 columns with ternary gradient mobile phases consisting of acetonitrile: methanol: 1% trifluoroacetic acid. The primary structures of oxytocin (OT) and vasopressin were confirmed by amino acid and microsequence analyses. A 210 nm absorbance peak was revealed with chromatographic properties closely related to those for OT, but without ME-activity. When injected into CD-1 male mice, 1.0 µg of the peptide significantly increased anterior pituitary DA at 15 and 30 min postinjection. Preliminary amino acid analysis revealed a Thr-rich decapeptide. Supported by N.I.H. grant #HD 19521.

506.11


Galanin (GAL) inhibits the breakdown of phosphoinositides (PI) stimulated by carbachol in minipsins from rat ventral hippocampus. Ca2+ influx promoted by depolarization or Bay K 8644, a selective agonist of the L-type voltage-dependent calcium channel (VSCC) prevented the inhibitory effect of GAL. Blockade of the L-type channel with nifedipine (nif) potentiated the inhibitory effect of GAL without affecting muscarinic stimulation of PI breakdown. Blockade of all VSCC with 200µM Cd2+ reduced muscarinic receptor mediated PI breakdown by 50% and prevented the inhibitory effect of GAL (10 µM). Cd2+ ions at 20µM concentration still abolished the GAL's effect. w-conotoxin, 2µM, which blocks the L- and X-types of VSCCs, by itself inhibited carbachol mediated PI breakdown by about 25% and when added before GAL it prevented the inhibitory effect of the peptide. The properties of the VSCC involved in the inhibitory action of GAL coincide with those described for the L-type VSCC (Research Council Grant 87.00031.44, Rome, Italy).

506.12


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506.10


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507.1

AMILORIDE-SENSITIVE SODIUM CHANNELS AND SALT PREFERENCE OF PREWEANING RATS. S.I. Sollars & J.L. Berensin, Dept. of Psych, Univ of Washington, Seattle, WA 98195.

A preliminary report (Evered, M.D., Proceedings Canadian Federation of Biological Societies Abstracts, 21, 1978) indicates that neurotensin (NT) causes an increase in water intake. We have replicated this experiment and extended this finding. We show that the action of NT does not occur independently of other neural pathways.

Long-Evans rats with lateral ventricular cannulas rapidly drank increasing amounts of water over the range of 72.9 fmol (1 ml) to 72.9 pmol (4.8 ml) of NT over a 30 minute period, with all latencies less than 5 minutes. Peripheral administration of 72.9 pmol of NT had no effect.

In order to test whether NT acted in concert with other neural pathways, animals were pretreated with receptor blockers in dosages that were 100 times the saturating molar concentration of NT (59.78 pmol). NT drinking was still abolished by atropine, curare and gallamine, suggesting the presence of PACAP27-NH2. However, the final yield of PACAP27-NH2 was only 1/10th that of PACAP. PACAP27-NH2 was also synthesized and found to exhibit a similar AC activating potency as PACAP. It is noteworthy that there are two forms of PACAP are present in the hypothalamus. A broad range of physiological and morphological experiments are now in progress to elucidate the physiological roles of PACAP27 and PACAP27-NH2. (Supported in part by NIH grants DK90049 and DK30167.)
SODIUM APPETITE IS NOT ENHANCED DURING LACTATION IN RATS. Edward M. Snipker, Joseph G. Verbalis, and Edda Thiel, Departments of Behavioral Neuroscience and Medicine, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Fifty years ago, Richter and Barile reported a small but statistically reliable increase in the intake of 3% NaCl solution by lactating rats maintained on "cafeteria" diet (Endocrinol., 23:15, 1938). However, in recent experiments we found no difference in the intake of 0.5 M NaCl solution between 50 lactating and 50 nonlactating female rats when they were maintained either on sodium-rich (NaD) or sodium-deficient (NaD) diets. Sodium losses in urine virtually disappeared in lactating rats, whereas in nonlactating rats the concentration in milk was unchanged and their pups (B/llter) grew normally. To determine whether lactating rats are able to develop sodium appetite, they were injected subcutaneously with polyethylene glycol (PEG) solution (5 ml), to reduce plasma volume isomotically, 8 days postpartum. The PEG treatment rapidly enhanced NaCl intake to levels above those of PEG-treated virgin NaD rats, and both NaD groups consumed more NaCl than did lactating and virgin NaD rats. These observations indicate that enhanced NaCl intake is not an integral feature of lactation despite the substantial sodium loss during milk transfer to the young, the marked increase in renal sodium conservation, and the evident ability of lactating rats to experience sodium appetite appropriately in response to a pronounced, acute need. (Supported by NIMH research grant MH-25140.)


We have reported that rats with ibotenic acid (IBO) lesions of the lateral parabrachial nucleus (LPBN) ingest increased amounts of water after peripheral or intravenous (i.v.) injection of angiotensin II or isoproterenol (Hendry, H. P. and A. K. Johnson. J. Comp. Physiol. 117: 593, 1977; Hendry, H. P. and A. K. Johnson. J. Comp. Neurol. 204: 149, 1982). However, lesions of the LPBN do not produce overdrinking. Thus, it is possible that the LPBN is not necessary for overdrinking, but that some other area outside the LPBN is involved. To determine whether the LPBN is necessary for overdrinking, we examined the distribution of HRP labeled fibers in lesioned and unlesioned LPBN and unlesioned control rats. When we compared the distribution of labeled fibers in lesioned and unlesioned LPBN, we found that passage remain intact after IBO lesions. (Supported by NIH HL14388)

ANATOMICAL BEHAVIORS VI FRIDAY AM
ROLE OF CENTRAL α2-ADRENOCEPTORS ON THE DIPSOGENIC EFFECT OF ANGIOTENSIN II. P. Colombo

Intraeardrenergic (IVC) injection of angiotensin II (AL) in rats increases the blood pressure (BP), heart rate (HR) and water intake (WI). The catecholamines of the central nervous system (CNS) are involved in these effects. We studied the effects of the α2- and α1-adrenoceptors on the pressor, tachycardic and dipsogenic action of AL injected intracerebroventricularly (IC). ICV injection of (12 ng) produced an increase in BP, HR and WI. Previously ICV treatment with α2-adrenoceptor agonist, clonidine (20, 40, 80 and 120 nmol), decreased the pressor, tachycardic and dipsogenic effects of AL. The α1-adrenoceptor agonist, prazosin (80 and 120 nmol) injected previously also reduced the pressor and tachycardic effect of AL, but not the dipsogenic response. The data confirm the role of central adrenergic system in the mediation of thirst and cardiovascular responses induced by AL. They also suggest that the central α2-adrenoceptors is involved only in cardiovascular responses produced by central AL, whereas all actions of AL can be modulated by the α2-adrenoceptors.

Research supported by FAPESP (88/9186-6).


We first showed that as little as 43 pmol (50 ng) CCK-8 injected into the LV suppressed chow intake for up to 1 hr and returned. CCK-8 alone decreased feed intakes throughout the 3 hr period (p<0.05); but with the combination of CR1409 and CCK-8, the suppression of feeding was reversed. In the fasted sheep CRI409 alone did not significantly increase feeding. CR1409 receptors, the satiety effects of CCK in the brain can be reversed. CRI409 was supplied by Rotta Research Laboratorium. Research supported by NIH NS20000.

EFFECT OF CCK ON FOOD INTAKE AND BEHAVIOR IN RATS. S. Wargish, Dept. of Pharmacology, School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1 and V.A. Medical Center, Minneapolis, MN 55417.

CCK's satiating effect may be due to gastric distension, secondary to inhibition of gastric emptying. To evaluate the interaction between distension and CCK on food intake and behavior we observed sleeping, resting, exploring, moving, grooming and feeding behavior in 24 hr food deprived rats following saline or CCK (5µg/kg) ip and intubation of a 1.5% guar test meal. Of 1.5, 3 & 6 ml). The rats were observed in their home cages with no Chow or a novel object present. CCK increased sleeping (132%) and eating (31%) and decreased grooming (28%) and resting (33%) compared to the saline group when chow was present, p<0.05. In the presence of a novel object, CCK decreased exploratory eating (88%) and chewing (80%) and increased sleeping (98%), p<0.05. CCK and distension did not interact to affect any behavior in the presence of Chow or a novel object. Without Chow present, CCK + test meal increased intakes 139% compared to the saline group and there was an interaction between CCK and gastric volume on this behavior. p<0.05. CCK had a satiating effect in rats in the presence or absence of food.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

INGESTIVE BEHAVIORS VI
FRIDAY AM

507.15

Intraperitoneal (ip) injections of bombesin (BBS; Gibas et al., 1979) and mammalian BBS-like peptides (e.g. GBP-10, DNPvTa and Gibas, 1985) reduce food intake in rats, suggesting that this peptide family may play a physiological role in satiety. We tested whether the intravenous (iv) infusion of BBS would produce similar effects. METHODS: Adult male Sprague Dawley rats with chronic inferior vena cava catheters were maintained on solid food. At 1000, rats were food deprived; at 1050 they were given an iv infusion of BBS (0.8, or 16 µg/kg) or equivolumetric vehicle control (1 ml/kg). Liquid food (BioServ, 40% v/v) was offered and intakes measured at intervals during a 150 min test. RESULTS: BBS reduced intake in a dose-dependent fashion. Percent reductions at 30 and 150 min were:

<table>
<thead>
<tr>
<th>Dose</th>
<th>30 min</th>
<th>150 min</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 µg/kg</td>
<td>35</td>
<td>15</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>16 µg/kg</td>
<td>42</td>
<td>24</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>16 µg/kg</td>
<td>67</td>
<td>39</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion: Intravenous infusions of BBS produce large, dose-dependent reductions in food intake. Thus, a local site of action within the abdomen, accessed by the ip route, is not required for the satiety effect of exogenous BBS in the rat. Supported by NIH grant DK33248 (JG).

507.17
CENTRAL ALLOXAN TREATMENT DECREASES THE INHIBITION OF FEEDING INDUCED BY NALOXONE AND CHOLECYSTOKININ IN RATS. D. Arquie and R.J. Bodnar. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

While peripheral alloxan destroys pancreatic beta cells and thereby produces diabetes, central alloxan (100 µg, ICV) reduces 2-deoxy-D-glucose (2DG) hypoglycemia without altering hyperglycemia, presumably by altering either brain glucoreceptors or a glucoregulatory control mechanism. The present study evaluated whether central alloxan altered the inhibition of intake of male rats deprived of food for the previous 24 h by the opiate antagonist, naloxone (NAL: 0.01-10 mg/kg, ip) or the peripheral satiety peptide, cholecystokinin octapeptide (CCK: 1-8 µg/kg, ip). Both NAL and CCK significantly inhibited feeding at 2 h, but not at 24 h after refeeding of food in vehicle-treated rats. Peak inhibition of food intake by NAL (10 mg/kg) was significantly reduced following alloxan (41% inhibition) vs. vehicle (62% inhibition) treatment. Alloxan shifted the ED50 of CCK inhibition to the right, but only reduced the peak inhibition effect of NAL. Central alloxan can therefore alter neurochemical systems producing inhibitory (nal and CCK) as well as stimulatory (2DG) effects upon food intake.

507.19
EFFECTS OF CHOLECYSTOKININ, LIGAND AND HYPERTROPIC NaCl SOLUTION ON GASTRIC ACID SECRETION IN RATS. Loreta M. Flanagan, Joseph G. Verbalis, and Edward M. Sanders. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Treatments with cholecystokinin, LIG and hypertonic NaCl solution each are known to decrease food intake, inhibit gastric motility, and stimulate oxytocin secretion in rats. Because the descending projections of the paraventricular nucleus of the hypothalamus (PVN) have been implicated in the control of feeding and gastric motility, it has been hypothesized that the PVN coordinates this array of responses to these treatments. One might further expect gastric acid secretion to be co-regulated with feeding and gastric motility because electrical stimulation of PVN known to increase acid secretion. To test that hypothalamic animals were fitted with gastric cannulae and acid secretion was measured hourly in awake, freely moving animals for 4 h after each treatment (n=5 for each group). After injection of cholecystokinin (0.1, 1.0, 10 µg/kg), there was a dose-related increase in gastric acid secretion (183%, 230%, 259% of control, respectively). In contrast, LIG (0.75, 1.5, 3.0 µg/kg) decreased gastric acid secretion (122%, 119%, 75%, respectively). In other groups of animals, gastric acid secretion was not correlated well with their effects on food intake, gastric motility, or pituitary oxytocin secretion.

507.20

Duoanal infusions of 5 kcal of Intralipid (IL) in sham feeding rats significantly reduce intakes and elicit behaviors typical of satiety (Greenberg et al., 1985). To determine whether these effects are mediated at preabsorptive sites we examined the time course for absorption of IL into the systemic circulation under test conditions identical to those for intake measures. Rats (n=7) were fitted with gastric cannulas and duoanal and inferior vena cava catheters. Rats were given 1.6 ml of [1-3C]-IL at a concentration of 0.5 kcal/ml. All [1-3C]-IL was given in the first 2 h with a total volume of 10 ml IL. A total caloric load of 5 kcal was delivered at a rate of 0.4 kcal/min. The time course of the appearance of [1-3C] in 100 µl plasma is shown below:

<table>
<thead>
<tr>
<th>Time of injection</th>
<th>Maximal effect Appearance in Plasma: 30 min 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>70% 15%</td>
</tr>
</tbody>
</table>

Satiation is therefore associated with the satiety associated with [1-3C] in plasma. This result lends further support for a physiological role for endogenous CCK in satiety.

Supported by USPHS NIMH grant 40010 (GPS).

507.16
EFFECT OF PYLORECTOMY ON THE INHIBITION OF GASTRIC EMPTYING BY CHOLECYSTOKININ (CCK) Timothy H. Moran, Robert J. Crosby, and Paul R. McHugh. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Removal of a population of type A CCK receptors localized to the circular muscle layer of the pyloric sphincter by pylorectomy results in a significant attenuation of the ability of CCK to inhibit gastric emptying. The present experiment addresses the role of CCK's inhibition of gastric emptying in the mediation of these results. The gastric emptying of 10 ml of saline or glucose (1.25 gm/ml) from the rats stomach following IP injections of 2 or 8 ug/kg or saline vehicle was quantified by the serial test meal dye dilution technique. Following acquisition of baseline data, rats were pylorocotomized, surgically removing pyloric CCK receptors and surrounding tissue. Two weeks following the pylorectomy procedure, at a time when the inhibition of food intake by CCK is significantly attenuated, the effect of CCK on the gastric emptying of saline and glucose test loads was reassessed. The results showed that postoperatively, CCK inhibited the emptying of saline and glucose test meals in a dose dependent manner. Pylorocytoma had no effect on CCK's ability to inhibit the emptying of saline test meals but attenuated the inhibition of the emptying of glucose. While demonstrating some role for pyloric CCK receptors in the inhibition of gastric emptying by CCK, these results suggest that this inhibition is complex and may depend upon multiple sites of action and mechanisms. (Supported by DDK19302)

Recently, our laboratory has demonstrated that very small doses (0.003 - 0.01 mg/kg) of DOI or D-lysergic acid diethylamide (LSD) increased REM sleep in rats. Since 5-HT-2 agonists have been reported to be hallucinogens, we were interested whether DOI might alter time perception in rats. In the present study, we report that 5-HT-2 agonists exerted no effects on time perception in rats, but no dose-response studies have been reported to obtain more accurate estimates of the perceptual midpoint of the light duration interval bounded by 1.0 and 2.5 sec and of the discriminability of these two stimuli.

No effect was found after low doses (0.01-0.1 mg/kg) of DOI. Both the 0.375 and 0.75 mg/kg doses appeared to increase the bisecion point in a dose-dependent manner. Discriminability declined as the dose increased, and a bias in reporting "short" was seen after these doses of DOI. There were no apparent biases in the bisecion function of dose after AMP (0.75 and 1.5 mg/kg). Discriminability declined as the dose increased, but no response bias was seen. The highest doses of DOI (1.5 mg/kg) induced erratic behavior, so these doses were not analyzed.

ANÁLISIS DE LA TEMORAL SUPERIOR DE PUP URSALANDS Y LA APLICACIÓN COMO MODELO PARA ANSIEDAD DISORDER. J.Mont* J.van Logten* A.J.Wolthuis*. C.N.S.-Pharmacology, Duphar b.v., P.O.Box 2, 1380 AA Weesp, Holland.

Rat pups emit ultrasonic calls at 35-50 kHz when they are separated from their mother and littermates. Several external factors influence this behavior, e.g., temperature, odours, bedding as well as endogenous variables, like age and duration of separation.

In a number of experiments the temporal structure of these calls was investigated. In the present study, we report that the ultrasonic environment markedly changed the structure of ultrasonic calls. In the absence of repeated bursts of repeated calls were observed. At low temperatures (18°C) whereas at 37°C more isolated calls were observed.

Serotonin reuptake blockers (fluvoxamine), 5-HT1a agonists like buspirone and benzodiazepines affect not only the number of ultrasonic calls, but also the duration in time. Many drugs do not affect these calls. On the basis of these results it seems possible to classify differential actions of various drugs effective in different forms of anxiety disorders.


Previous research suggests that serotonin exerts an inhibitory influence on food intake. Results using microdialysis demonstrated that extracellular serotonin increased in the perifornical region of the lateral hypothalamus (PHF) in conjunction with a meal or injection of peripheral or local 5-7-2-serotoniner.

In the present study, the anorectic potency of lateral hypothalamic serotonin was investigated during the three-hour period following central injections in mildly food-deprived rats. Serotonin (0.2, 0.5, and 1.0 mmol/kg) was injected in the PHF produced a dose-dependent reduction in food consumption at 15 and 30 minutes post-injection (*p<0.002). No differences in cumulative intake were detected after 60 minutes at the doses tested. The suppressive effect of the serotonin (25 nmoi) 30 minutes post-injection was found to be significantly attenuated by pretreatment with systemic injection of the serotonin antagonist metergoline (1 mg/kg), but not by the dopamine receptor antagonist haloperidol (0.25 mg/kg). These results support the hypothesis that serotonin transmission in the PHF contributes to the inhibition of feeding behavior.

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TM is a widely practiced mental technique that results in improved health and adaptation to stress. Previous research has documented an acute attenuation of the SNS during TM, but no direct investigation of acute PNS effects has been reported. Also the acute effects of TM on the monoamines, serotonin (5-HT) in particular, remain to be clarified. We used a combination of heart rate variability (HRV), respiratory sinus arrhythmia (RSA) and heart rate variability (HRV) as an indirect measure of cardiac vagal tone during TM. Skin Resistance (SR) was employed as an indicator of SNS tone. The results were that during TM, particularly at times of spontaneous respiratory apneas that are known to mark the subjective experience of "transcending", HRV was almost absent and SR showed no change. These results indicate that both PNS and SNS tone were attenuated. In a second experiment we measured changes in platelet 5-HT content (HPLC-ECD). These results were consistent with the hypothesis of an acute reduction in central and peripheral 5-HT activity during TM practice. There was a positive correlation (r=0.49, P=0.05) between the "High-Driving and Competitive" subscale of the Buss-Durkee Competitive-Competitive Scale and the HPLC-ECD results of the platelet serotonin (5-HT).

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The 5HT1a agonists 8-OHPDAP and 5MEODMT exert antidepressant-like actions on the DRL 72 sec schedule (increased response rate). Buspirone, gepirone and ipsapirone are also potent 5HT1a agonists. Since buspirone and gepirone have recently been shown to block the 5HT1a receptors in guinea pigs, we evaluated their effects on the DRL schedule. Buspirone and gepirone (25-5 mg/kg) produced increases in response rate. Ipsapirone did so only at 20 mg/kg. 8-OHPDAP and 5MEODMT also showed AD-like effects, as previously shown. The potent 5HT1a antagonists BMY 7378 and NAN-190 antagonized the AD-like effects of buspirone and gepirone but were ineffective against 8-OHPDAP or 5MEODMT. The opposite pattern was observed with methysergide, which antagonized the effects of 8-OHPDAP and 5MEODMT but which was ineffective against buspirone and gepirone. (-)-Propranolol, an antagonist at 5HT1A and B adrenergic receptors, gave mixed results. It is concluded that 1) consistent with their clinical activity, buspirone and gepirone act as antidepressants on a behavioral screen, 2) their mode of action differs from that of 5MEODMT and 8-OHPDAP, and 3) there may be multiple populations of 5HT1A receptors. (Supported by NH 11191 and 10962 to LSS)

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SEROTONERGIC INVOLVEMENT IN DOPAMINE-DEPENDENT CATECHOLAMINEERIE RESPONSES IN THE RAT. B.S. Neal, J. Lucki and J.N. Joyce, Departments of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Antipsychotic-induced catalepsy is primarily associated with dopamine (DA) receptor blockade. Recent reports suggest that central serotonergic activity may have a significant influence upon this type of catalepsy. Our aim was to examine the ability of several dopaminergic compounds, including selective D1 (SKF-38393) and D2 (quinpirole) receptor agonists, to block catalepsy. We also examined the ability of 5HT agonists to affect antipsychotic-induced catalepsy, using agents which act selectively at the various 5-HT receptor subtypes.

The dopaminergic compounds, apomorphine (0.1-3.2 mg/kg), d-amphetamine (1.0-5.6 mg/kg), and quinpirole (0.1-3.2 mg/kg) were all effective, at certain doses, to reverse haloperidol-induced catalepsy. SKF-38393 (0.1-3.2 mg) had no significant effect.

The following serotoninergic agents, at certain doses, also reversed catalepsy: 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT). BMY 7378 (1.0-1.6 mg/kg), and DOB (0.1-3.2 mg/kg). Comparisons between dopaminergic and serotonergic agents were also conducted by examining shifts in the dose-effect curves for haloperidol-induced catalepsy.

These results suggest that not only is antipsychotic-induced catalepsy caused by DA D2 receptor blockade, but there is also a significant serotonergic influence, involving more than one of the 5-HT receptor subtypes. Studies are currently underway to further elucidate how and where this serotonergic influence, involving more than one of the 5-HT receptor subtypes, contribute to catalepsy.

This research was supported by U.S.P.H.S. grants MH48564 (B.S.N.), MH43652 (J.N.J.), MH02652 and GM34781 (J.L.)

508.10


Both systemic and intra-amygdala injections of the 5-HT3 receptor antagonists GR80323F, MDL72292 (MDL), ICS265-930 (ICS), BRL43694 (BRL) and GR65657 (GR65) into the rat increase social interaction (SI) under conditions of maximal isolation, is high light/unfamiliar (HL). After intra-amygdala injections of GR80323F (100mg), MDL (100mg), ICS (100mg), BRL (100mg) and GR65652 (10mg) the predominant behaviors involve changes to nose sniffing, following & grooming of the untreated partner rats. Individual bout frequency & duration were also increased. These behaviors were similar to those induced in control rats by environmental manipulation, i.e. low light/familiar (LF) compared to HL conditions, indicating that they reflect a reduced anxiety state. Conversion of the rat's preference for SI to preference for the untreated partner rats, increased. These findings provide evidence that the disinhibitory effects of 5-HT receptor antagonists in the rat SI test are mediated at least in part via the amygdala.

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Although cocaine potently inhibits serotonin uptake, the contribution of this property to cocaine's central activity is unclear. In a previous study, serotonergic systems, activated by treatment of C57 BL/6J mice with serotonin (5HTP), a potent and selective serotonin uptake inhibitor, 60 mg/kg ICV, were blocked by cocaine (COC). Opiates, such as phencyclidine, Mossbauer, 10 mg/kg, i.p., did not change the locomotor activity measured for 50 min after injection of COC (25 mg/kg, i.p.) on saline or male C57 mice. Pretreatment with 8 mg/kg of PH or with placebo resulted in identical dose-response curves for COC (10-40 mg/kg), indicating that the lack of effect of SER was not due to a ceiling effect. Although SER did not potentiate or attenuate the overall locomotor response to COC, there was a shift in the response towards lower doses. At 16 and 32 mg/kg of SER, both in male and female mice. This was probably not due to a pharmacokinetic interaction between the compounds, because pretreatment with SER (16 or 32 mg/kg) did not affect the plasma and brain concentration of COC measured (by HPLC) 12 min after injection of COC (25 mg/kg) when brain levels of COC were maximal. The SER-induced delay of the locomotor response to COC is consistent with previous hypotheses of an inhibitory modulation by serotonin of COC's locomotor and rewarding effects. (Supported by PfizerPharmac., #86-N-012)

508.12

STRESS AS THE CAUSE OF SEROTONERGIC DYSFUNCTION IN MENTAL DISORDERS: SUPPORT FROM CORRELATES OF SEROTONIN TURNOVER IN NORMAL SUBJECTS. K.G. Walton, S. Goluboff* (H.DQ. Pagli*, M.L. McGee*, D. Goodall*, E. MacLennan and B. Levitsky*). Neurochemistry Laboratory, Departments of Chemistry and Psychology, Maharishi International University, Fairfield, IA 52556.

Several serotonergic activities, reduced in a variety of personality and affective disorders. Reduced serotonin turnover has also been reported in depressed subjects, even on the whole-body level. We investigated excretion of the metabolite, 5-hydroxyindoleacetic acid (5HIAA), an accessible indicator of whole-body serotonin turnover, in relation to standard tests of personality and affect in normal subjects. In the first study, 5HIAA excretion in 33 women students (but not men) correlated negatively with a measure of neuroticism and depression (r = -.47 and -.51, P < .005). In a second study, 5HIAA correlated negatively with aggression (r = .43, P < .005) and impulsiveness (r = .28, P < .05) only in men. These correlations and sex differences persisted in a low-scess group. The low-stress group also showed higher nighttime serotonin turnover (P < .0005, df = 52) and a day-night difference in turnover (night higher) not found in the average-stress group. These differences and sex differences persisted in a low-stress group. The low-stress group also showed higher night-time serotonin turnover (P < .0005, df = 52) and a day-night difference in turnover (night higher) not found in the average-stress group. These differences and sex differences persisted in a low-stress group. The low-stress group also showed higher night-time serotonin turnover (P < .0005, df = 52) and a day-night difference in turnover (night higher) not found in the average-stress group. These differences and sex differences persisted in a low-stress group. The low-stress group also showed higher night-time serotonin turnover (P < .0005, df = 52) and a day-night difference in turnover (night higher) not found in the average-stress group. These differences and sex differences persisted in a low-stress group.
SEROTONERGIC DORSAL RAPHE NEURON ACTIVITY RELATED TO FEEDING/GROOMING BEHAVIORS IN CATS.


The discharge activity of central serotonergic neurons has been shown so far to basically vary in association with behavioral states. Serotonergic cells were recorded using methods described previously (Formal et al., Exp. Neurol. 98: 338-403, 1987). Unit activity was monitored during spontaneous feeding/grooming behaviors and during sleep. In addition to highly regular firing DRN serotonergic neurons, we found cells that discharged in a somewhat regular manner during quiet waking (N=9, 4 spks./N=6) or during REM sleep (N=12) and completely off during REM sleep and in response to systemic administration of serotonin agonist drugs. These cells displayed a firing rate of 5.3±1.1 spks/ (N=7) during feeding-related movements (e.g. licking) and 5.9±1.4 spks/ (N=6) during grooming-related movements. Of those that were monitored for 1 hr after waking, their firing rate was similar to or less than that during quiet waking. Our results indicated that serotonergic neurons may modulate target structures in association with specific behaviors, as well as, the general level of behavioral arousal.

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LONG-TERM (24 HR) ENHANCEMENT OF THE SENSORY-MOTOR CONNECTION MEDIATING TAIL WITHDRAWAL REFLEX IN APLYSIA. F. Noel*.

D.V. Buonomano and J.H. Byrne. Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77225.

Previous work has demonstrated that a cellular analogue of differential classical conditioning protocol produces a short-term (min) form of associative plasticity term activity-dependent neuromodulation in the connections between sensory neurons (SNs) and motor neurons (MN) (Scholz and Byrne, 1987; Walters and Byrne, 1983). EPSPs produced by SNs whose activity was paired with a reinforcing stimulus is significantly larger than in control conditions. We have now extended this observation by examining long-term (24 hr) enhancement of EPSPs produced by a similar training protocol.

Two SNs in the pleural ganglia, which synapsed onto a common MN in the pedal ganglia, were fired 3 times with a 5 sec interspike interval to establish baseline EPSPs. Training consisted of 8 trains of shocks delivered to the body wall of the animal over a 1.5 hr period. The connections between the SNs and MNs were examined before, 5 min after, and 24 hr after the training procedure by firing a SN with a brief intracellular depolarizing pulse and recording the monosynaptic EPSP in the MN. The mean amplitude of the EPSP measured 24 hr after training was significantly greater than (p<0.001, n=8) the control at 24 hr after training. The mean input resistance of the MN maintained in organ culture for the 24 hr period between recordings.

The mean amplitude of the EPSP produced by this training was significantly larger than (p<0.05) the control at 24 hr after training. The mean amplitude of the EPSP measured 24 hr after training was significantly greater than (p<0.001, n=8) the control at 24 hr after training. The mean input resistance of the MN maintained in organ culture for the 24 hr period between recordings.

Both the short-term (min) and long-term (24 hr) enhancement in EPSP amplitude did not appear to be the result of associative plasticity term activity-dependent neuromodulation in the connections between sensory neurons (SNs) and motor neurons (MN) (Scholz and Byrne, 1987; Walters and Byrne, 1983). EPSPs produced by SNs whose activity was paired with a reinforcing stimulus is significantly larger than in control conditions. We have now extended this observation by examining long-term (24 hr) enhancement of EPSPs produced by a similar training protocol.

Intracellular injection of cAMP produces an increase in food intake in free feeding rats. This action is thought to be mediated via an increase in feeding-related movements (e.g. licking) and 5.9±1.4 spks/ (N=6) during grooming-related movements. Of those that were monitored for 1 hr after waking, their firing rate was similar to or less than that during quiet waking. Our results indicated that serotonergic neurons may modulate target structures in association with specific behaviors, as well as, the general level of behavioral arousal.

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Differential effects of the peptide buccalin and serotonin on membrane currents, action potential duration, and excitation in hermissenda type B photoreceptors.


Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Buccalin is a neuropeptide isolated from the buccal mass of Aplysia (Cropper et al., 1988). Since it is distributed throughout the CNS, it affects membrane properties of sensory neurons (SNs) in the cerebral ganglion (Rosen et al., 1989), and we looked for effects of buccalin on membranepotentials (Forrester and Crow, 1988). Clusters of SNs from the pleural ganglia were isolated and maintained at 20°C in ASW. Spike duration was determined by eliciting single spikes with 3 sec depolarizing current pulses, and excitability was measured as the number of spikes elicited by a 1 sec depolarizing current pulse. With 5 and 9.5 sec test pulses, buccalin (50 nM) applied to the static bath significantly increased excitability (n>9). In the same preparations, buccalin produced a modest increase in spike duration that was not statistically significant. H-7, which increases both excitability and spike duration, was subsequently applied, spike duration increased dramatically, but there was no further significant increase in excitability.

In tail SNs, the effect of 5-HT on excitability is due primarily to a CAMP-dependent decrease of the S-current (Ia), whereas its effect on spike duration is primarily the result of a CAMP-independent participation of the delayed K+ current (Ih). In order to determine which currents might be mediating the effects of buccalin, two-electrode voltage clamp experiments were performed. At 15°C, buccalin decreased an outward current whose linearity is characteristic of Ia. At 30°C, buccalin increased the magnitude of Ia but had no effect on Ih.

As a first step in the investigation of the neural circuitry underlying the expression of behavioral changes associated with conditioning in *Aplysia*, four light-sensitive cell types were identified from intracellular recordings of photoreceptor and the pedal ganglia. Results using synaptic blockers and anatomy of the optic nerve showed that the light responses are not intrinsic to the pedal cells and suggested that the pedal cells’ light responses are due to synaptic input from the photoreceptors (Hodgson & Crow, 1987). However, extracellular photoreceptors may contribute to the light responses of the identified pedal cells. To determine if the pedal cells receive input from other light responsive neurons whose cell bodies or axons may have been cut or damaged during the anatomy experiments, the light response of each pedal cell type was recorded before and after the eyes were removed by a suction technique that left all other nervous system structures intact. The light responses of the four cell types were abolished after eye removal, confirming the results of previous experiments using synaptic blockers and anatomy.

The anatomy and light responses of the identified pedal cells have also been further characterized. Lucifer yellow cell fills show that all four cell types have one process that exists the nervous system via a identified pedal nerve. Cells P7, P9 and P10 exit in nerve P5; P7 exits in nerve P1. Intracellular recordings of pedal cell responses to 5-10-minute light steps show that P7, which has an inhibitory light response, shows a decrease in spike frequency during the light step (p < 0.05). P8 showed no significant change in spike frequency during a long light step, however this neuron does exhibit an excitatory response to light.

SENSITIZATION IN VIVO: MASKING OF AN OCCULT FACTORIAL EFFECT OF CONNECTIVE STIMULATION. T.G. Nolen and C. Billeret. Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124 and the Marine Biological Laboratory, Woods Hole, MA 02543.

Nervous stimulation produces several forms of non-associative learning in *Aplysia* california. In adults, a shock to the tail produces two apparently sequential effects on the siphon withdrawal reflex – initial reflex inhibition followed by sensitization some 30 minutes later (Marcus et al., '88 SCI 241:210). During ontogeny, sensitizer neurons emerge during the juvenile development (Rankin & Carew, 98, J. Neurosci. 8:197; Nolen & Carew, 98, J. Neurosci. 8:321).

The late emergence of sensitization may reflect the delayed maturation of the facilitatory process and/or the inhibition of an emerging, but weak form of sensitization. To test the latter possibility, we investigated the ability of connective stimulation (an analog of tail shock) to produce inhibition and facilitation of the siphon nerve evoked EPSP in R2. We used phophodiesterase inhibitors (e.g. IBMX) to enhance the facilitatory effects of connective stimulation by elevating the levels of cAMP in early stage 12 juveniles, a time when sensitization is emerging.

Most early stage 12 preparations showed inhibition, rather than facilitation. Early to Mid stage 12 preparations were assigned to one of two classes, NON-FACILITATED or FACILITATED, depending on the effect of connective stimulation on the evoked, complex EPSP. In NON-FACILITATED preparations the light responses were abolished after eye removal, confirming the results of previous experiments using synaptic blockers and anatomy.

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SENSORY NEURONS IN VITRO DUE TO 5-HT RESEMBLE THE ROLE OF PRIMITIVE VISUAL PATHWAYS, THE OPTIC NERVE IN A P L Y S I A. T.M. Hodgson and T. Crow. Dept, of Neurobiol. and Anal., Univ. of Texas Med. Sch., Houston, TX 77225

As a first step in the investigation of the neural circuitry underlying the expression of behavioral changes associated with conditioning in *Aplysia*, four light-sensitive cell types were identified from intracellular recordings of photoreceptor and the pedal ganglia. Results using synaptic blockers and anatomy of the optic nerve showed that the light responses are not intrinsic to the pedal cells and suggested that the pedal cells’ light responses are due to synaptic input from the photoreceptors (Hodgson & Crow, 1987). However, extracellular photoreceptors may contribute to the light responses of the identified pedal cells. To determine if the pedal cells receive input from other light responsive neurons whose cell bodies or axons may have been cut or damaged during the anatomy experiments, the light response of each pedal cell type was recorded before and after the eyes were removed by a suction technique that left all other nervous system structures intact. The light responses of the four cell types were abolished after eye removal, confirming the results of previous experiments using synaptic blockers and anatomy.

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RULES FOR BEHAVIORAL CHOICE IN APLYSIA FASCICATA
Aplysia has been used by many researchers to study the neural control and mechanisms involved in behavior.

1. Animals can be engaged in a pleural sensory neuron, which is a component of the CNS, and has been shown to be capable of generating action potentials (APs) in response to various stimuli. The APs can be affected by neuromodulators, such as Conopressin G, which can increase or decrease the duration of the APs.

2. Conopressin G, a peptide of the vasopressin-oxytocin family, has been shown to affect the duration of the sensory neuron action potential (AP) in a dose-dependent manner. The increase in AP duration is specific to Conopressin G and does not occur in response to other peptides.

3. The change in AP duration is dependent on the concentration of Conopressin G. A low concentration of Conopressin G increases the AP duration, while a higher concentration decreases it.

4. Conopressin G affects the sensory feedback from the ovotestis, which is needed in controlling the ventilatory rhythm in the abdominal segments. Therefore, the sensory feedback from the ovotestis is needed in the development and evolution of segmentally homologous abdominal segments.

The Peptide Modulation of Motor Output during Egg-Laying Behavior in the Snail Lymnaea stagnalis is characterized by a stereotyped behavior lasting 2-3 hours. This behavior is an all-or-none event that is triggered by a synchronous burst of activity in the central command (CCD), a group of neuroendocrine cells in the cerebral ganglia of the central nervous system. The overt egg-laying behavior of Lymnaea is comprised of three distinct phases: resting, turning, and oviposition, each named after the activity of the animal during these phases.

The typical patterns of motor activity of the head/foot and the buco-abdominal mass of the animal that occur during egg-laying behavior are carried by a coordinated action of sensory feedback from the ovotestis, and several peptide hormones and putative neurotransmitters that are released from the CCDs during the discharge into the blood and to the CNS, respectively.

We investigated the effects of one of these peptides, Caudo-Dorsal Cell Hormone (CDCH), on the motor output of the CNS. To this end, we have analyzed the locomotion of the animal during the different phases of egg-laying behavior using a video-digitizer. The motor output of the CNS was analyzed in vivo and in vitro using permanently implanted electrodes and a novel waveform recognition program. Our results show that application of CDCH modulates the motor output of the CNS. However, the sensory feedback from the ovotestis is needed to trigger the full complement of the behavior in vivo.

OPTICAL RECORDING OF NEURAL ACTIVITY: ABSORPTION SIGNALS FROM NEURONS INJECTED WITH VOLTAGE SENSITIVE DYES.

We investigated optical signals from individual neurons from Drosophila melanogaster in vivo using intracellular dye injection. Our aim is to find signals that are large enough to allow the analyses of the properties of individual neurons in the intact ganglia. We concentrate on absorption mode of measurements for two main reasons: 1) in terms of photodynamic damage fluorescence dyes are significantly worse than absorption dyes; 2) intracellular dye application results in high background fluorescence which reduces the signal to noise ratio.

Negatively charged oxonol dye RGA-509 that gave largest absorption signals after extracellular application did not produce any signal when injected into the neurons. Both fluorescence and absorption signals were obtained using positively charged styryl dyes RH-461 and RH-447: signal-to-noise ratio was 6 fold smaller in absorption. We are currently screening analogs of merocyanine-chodanine and merocyanine-oxozolone type for larger absorption signals.

INVERTEBRATE SENSORY SYSTEMS I

DEVELOPMENT AND EVOLUTION OF SEGMENTALLY HOMOLOGOUS SENSORY SYSTEMS IN THE LOCUST. M. Reiche*. Department of Zoology, University of Geneva, CH-1211 Geneva, Switzerland.

Our goal is to understand general organizational principles of the metamerie nervous systems. For this we are investigating the differentiation of a homonymously segmented embryonic nervous system into segmentally specialized adult nervous system. During the development of the thoracic and abdominal segments in the locust, identified clusters of mechanosensory neurons derive at characteristic positions along the body wall extradurally or laterally. Three parts of these sensory neurons differentiate as multicellular aggregates by epithelial invagination, cell migration and subsequent projection into the CNS. They give rise to the wing chordotonal organs in the thoracic segments, to the auditory organ in the first abdominal segment and to the pleural chordotonal organs in the remaining abdominal segments. In a dorsal cluster an identified neuron differentiates into the wing stretch receptor in the pterothoracic segments and becomes involved in controlling the ventilatory rhythm in the abdominal segments. Thus a number of adult sensory structures which are involved in completely different behavioral tasks are segmentally homologous and probably share a common evolutionary origin. Supported by the SNF.


We are examining the embryonic development of 3 groups of identified local interneurons in the locust. These neurons integrate sensory input from the legs and map this input as a set of overlapping receptive fields. The aim of this work is to identify the neurons that contribute to the morphological development of the interneurons. Using intracellular injection of cobalt we have observed the morphological development of the interneurons, which starts from the earliest stage at which they can be identified (50%), through to the first days of larval life. The development of the leg exteroceptors coincides with that of the interneurons.

Two NBs have been identified whose progeny form at least part of the complement of interneurons. Supported by BERC and a Royal Society Locke Fellowship to G.J.L.

INVERTEBRATE LEARNING AND BEHAVIOR IV FRIDAY AM
510.4


The hair-like sensilla on the legs of the blowfly, Phormia, can be assigned to one of three categories based on the neuropil region to which their axons project. A previous study showed previously that for the antennal ganglion, tactile and proprioceptive neurons were largely segregated into the ventral and dorsal neuropils, respectively. However, it is not yet known whether these patterns of projection are conserved across other fly taxa. We are investigating the afferent projections of leg sensilla across a range of fly species to determine if there are significant differences in the patterns of projection that may be related to differences in leg structure and function. The overall goal is to understand how the structure and function of the leg sensory system is conserved across different fly species.

510.5

PLURISEGMENTAL INTERNEURONS CARRYING ANTENNAL-DERIVED TACTILE SENSORY INFORMATION IN THE COCKROACH: J.A. Burdohan* and C.M. Comer (Spon: R. Ruth). Dept. Biological Sciences, Univ. of Illinois, Chicago, IL 60680.

The ventral giant interneurons of the cockroach, Periplaneta americana antennae, are known to be involved in escape behavior. Recent work in our lab has demonstrated that these interneurons carry sensory information from the leg mechanoreceptors. We have found that these interneurons are activated by mechanical stimuli applied to the cerci, which are the sensory organs located at the base of the antennae. The sensory input to these interneurons is mediated by the hair plate sensilla on the cerci, which are mechanoreceptors that respond to mechanical stimuli.

510.6

CONNECTIONS BETWEEN VENTRAL GIANT INTERNEURONS AND THORACIC INTERNEURONS OF THE COCKROACH OCCUR SPECIFICALLY ON THE VENTRAL MEDIAN BRANCH OF THE TIS. L.L. Stackbridge and A.E. French. Dept. of Biology, Univ. of Alberta, Edmonton, CANADA.

The thoracic interneurons of the cockroach, Periplaneta americana, are known to be involved in locomotion and escape behavior. We have found that these interneurons are connected to the ventral median branch of the thoracic intersegmental nerves (TIs). We have also found that the connections between these interneurons are specific, occurring only on the ventral median branch. These findings suggest that the ventral median branch is a critical region for integrating sensory information from the cerci and the thoracic intersegmental nerves.

510.7

ANATOMY AND PHYSIOLOGY OF IDENTIFIED CERICAL AFFERENTS IN THE COCKROACH. Daryl L. Daley. Department of Physiology, National College of Chiropractic, Lombard, IL 60148.

The cockroach has two pairs of cerci located at the base of the first and second antennae. These structures are involved in chemical and tactile sensing, and are thought to be essential for locomotion and orientation. We have found that the cerci of the cockroach are innervated by a single pair of cercal nerve fibers, which project to a defined region of the thoracic ganglion. We have also found that these fibers are sensitive to mechanical stimuli, and that they are activated by mechanical stimuli applied to the cerci.

510.8

ION CHANNELS IN ISOLATED MECHANORECEPTOR NEURONS FROM THE CONNECTIVE CHORDOTONAL ORGAN OF THE PEDICEL OF THE AMERICAN COCKROACH. L.L. Stackbridge and A.E. French. Dept. of Physiology, Univ. of Alberta, Edmonton, CANADA.

The American cockroach, Periplaneta americana, has a pair of large sensory organs located on the pedicel of the first pair of antennae. These organs, called the connective chordotonal organs (CCOs), are known to be involved in mechanosensory functions. We have isolated and studied isolated mechanoreceptor neurons from the CCOs to determine the role of ion channels in their function. We have found that these neurons express a variety of ion channels, including those known to be involved in mechanosensory transduction.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
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510.9


Experiments were performed to assess the role of a group of identified local interneurons (IIs) in the processing of wind information by the cricket cerebrocerebral sensory system. The stimulus-response characteristics of giant ascending interneurons (IIs) were measured by monitoring membrane conductances and responses evoked by photoinactivation of a subset of the local IIs. The activity of the giant IIs was monitored simultaneously using whole cell patch recording and multi-unit spike recording software. The results indicated that the local IIs have a functional excitatory influence upon giant IIs with similar directional sensitivities.

In simultaneous intracellular recordings of a local II and a giant II with similar directional sensitivities, we observed a depolarization of the local II caused by an increased excitability in the giant II. Computer reconstructions of these pairs of cells showed a high degree of dendritic overlap. Therefore it is possible that the observed excitatory influences are mediated by direct synaptic contact. Supported by NSF and NIH grants to JPM.

510.10


Directional sensitivities of wind-sensitive interneurons (IIs) in the cerebrocerebral system depend on aspects of their complex dendritic structure. To gain a more quantitative understanding of synaptic integration in these IIs, we have developed computer models using complex input impedance measurements. The anatomical parameters for the models were derived from stained IIs using computer reconstruction software. The resulting models were compared to experiments in which the complex input impedances of IIs were measured using a switched single-electrode current clamp. In some experiments, a laser was used to photophasically activate dendritic segments and the multi-unit spike responses were recorded. Such experiments allow determination of the distribution of membrane conductances in the model. The modeling calculations indicate that different parts of a single IIs are relatively independent in response to synaptic input, but different IIs are isolated from one another (and from the II) by significant complex impedances. These impedances strongly influence the relative weighting of synaptic inputs from sensory afferents of different directional selectivities. Supported by SRC/MATO (HB) and NSF (JT, NM & JM).

510.11

CHARACTERIZATION OF SYNAPTIC INTEGRATION BY ELECTROPHYSIOLOGICAL ANALYSIS AND MODELING. J. Pinto*, J. Tromp* and R. Nevins* Dept. of Zoology, Univ. of Calif., Berkeley, CA 94720.

Understanding the information processing of neurons in functional systems requires knowledge both of the synaptic and active membrane properties, and of the interaction of these active regions across the structure of the cell. An approach for analyzing remote synapses was suggested by Carnevale & Johnston (J. Neurophysiol 57:1700). Their two-port model is an anatomical conformer, and describes steady-state synaptic attenuation by purely electrophysiological means.

We are using this approach in conjunction with a precise compartmental model to study synaptic integration in an identified interneuron of the cricket cerebrocerebral sensory system. Electrophysiological measures fix parameters of the model, and the model in turn indicates the degree to which some of the approximations of the two-port analysis are correct. Thus the model constrains an interpretation of the physiological results by integrating various data into the true anatomical structure, and extends the power of the two-port analysis to nonideal conditions. (This work supported by an NSF grant to J.P. Miller, and NIH to JU.)

510.12


Regular IPSP or brief hyperpolarizing current pulse barrage effects on coding of injected excitatory sine currents were examined in the slow-adapting receptor (OSM). The post synaptic contribution was determined by comparing: (a) IPSP and pulse peak hyper- (H) and subsequent threshold depolarisation (T), and HT slopes; (b) times between an IPSP or pulse and the closest spike preceding it, or following, known as phase (ρ) and cophase (θ), respectively. Lockings, including 5 pulses or IPSPs and R spikes and driving at S/R times barrage rate, were over present. Jumps between S/R rates occurred at certain excitation values. Lockings showed: (a) gradual increase, decrease at sine depolarizing segments, respectively; (b) – increase and parallel control intervals and inhibitory minus control differences, respectively; (c) T were invariant, H and HT increased and decreased at sine depolarizing segments, respectively, and increased with barrage rate. IPSP and pulse effects were determined by H and HT, and were similar, therefore they were due to post synaptic properties. The functional meaning can only be conjectured about, but could provide greater control flexibility than the usually proposed summation.

* Supported by DECT grant to W.B.

510.13

ROLE OF SENSORY NEURONS IN BEHAVIORAL CHANGES DISPLAYED BY DEVELOPING CRAYFISH, E. N. Leise, N. A. Fricke and D. H. Edwards, Biol. Dept., Georgia State University, Atlanta, GA. 30303.

Stimulation of abdominal tactile afferents excite the LG command neurons for escape tailflip by two pathways: a monosynaptic electrical connection and a disynaptic pathway with respect to LG at rest, which forward at about 3 to 4 cm long, the disynaptic pathWeight is increased after photoactivation of a subset of the LG neurons. The activity of all the giant LGs was monitored simultaneously using whole cell patch recording and multi-unit spike recording software. The results indicated that the local LGs have a functional excitatory influence upon giant LGs with similar directional sensitivities.

In additional simultaneous intracellular recordings of a local LG and a giant LG with similar directional sensitivities, we observed a depolarization of the local LG caused by an increased excitability in the giant LG. Computer reconstructions of these pairs of cells showed a high degree of dendritic overlap. Therefore it is possible that the observed excitatory influences are mediated by direct synaptic contact. Supported by NSF and NIH grants to JPM.

510.14


We examined the properties of mechanosensory neurons in the first antennae of 10 species of pelagic calanoid copepods. Neural activity was recorded extracellularly from the base of the first antennae. Controlled mechanical stimuli were delivered with a vibrator driven by a waveform generator. The receptors responded to stimuli between 40 and over 1000 Hz. Unlike the previously described decapod mechanoreceptors, we found that sensitivity increased with frequency, and at 1000 Hz, threshold sensitivities were near 10 nm. Phase-locking of spikes to oscillatory stimuli was observed at frequencies up to 500 Hz.

Different species showed different types of neural responses. Responses in most were characterized by a large number of small spikes, where individual units could be identified as the base of the first antennae. Controlled mechanical stimuli were delivered with a vibrator driven by a waveform generator. The receptors responded to stimuli between 40 and over 1000 Hz. Unlike the previously described decapod mechanoreceptors, we found that sensitivity increased with frequency, and at 1000 Hz, threshold sensitivities were near 10 nm. Phase-locking of spikes to oscillatory stimuli was observed at frequencies up to 500 Hz.

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The biogenic amine, bistramine (BA), has been implicated to act in the olfactory CNS of arthropods (Maxwell et al., Comp. Biochem. Physiol., 61C:109, 1978) and annelids (Bhode et al., Neurosci. Abstr., 4F47-2-1989). We show that the olfactory lobe (OL) tissue of the spiny lobster is capable of synthesizing HA from its radioactive precursor, histidine, and that specific HA-like immunoreactivity (polyclonal antibody, Chemicon) occurs in: (1) cell body clusters of olfactory interneurons adjacent to the OL, (2) the outer cap region of the OL glomeruli, and (3) small cells, presumably glia, adjacent to the glomeruli that are possibly involved with NA uptake or inactivation. The distribution of MA in the OL glomeruli combined with recent evidence from our lab for a NA-gated Cl- channel on lobster olfactory receptor cells (McClintock et al. submitted) suggests that BA functions as an inhibitory neurotransmitter in the OL. Combined pharmacological and physiological experiments to test this hypothesis are in progress. (Supported by NSF Awards BNS 86-07660 and 88-10261.)

510.16 NEURONAL ORGANIZATION AND FUNCTION OF THE PECTAL SENSORY SYSTEM IN SCRIPIONS, H. R. Brownell. Dep. of Zoology, Oregon State University, Corvallis, Oregon 97331.

The pectines are large, sexually dimorphic appendages located on the mid-ventral surface of all scorpions, primitive (Silurian, aquatic and modern terrestrial). Behavioral observations of Paruroctonus mesaensis indicate the pectines are contact chemoreceptors of general sensitivity and probably function in male scorpion social behavior. The pectinal sensory organs are arranged in discrete patches that together form an organized, two-dimensional array of approximately 10 sensory neurons. Electrophysiological recordings from single pectinal sensory neurons show that each contains one mechanoreceptor and several chemoreceptors that respond to aqueous and nonpolar solutions applied to the sensillum tip. Cobalt infusion and intensification of chemo-receptor axons revealed a central projection that is topographically organized and sexually dimorphic in 5 species of 6 extant scorpion families. This organization is confirmed by degeneration studies showing topographic ordering of synaptic terminals in glomerular and layered neuropil. Based on these findings and other behavioral and biochemical studies, we hypothesize that the primary function of the pectal sensory system is to guide orientation to chemical signals borne on the substrate. Supported by NSF-BNS 8709890


Using ultramicroscopy, an ultrastructural examination of the cuticle of the various eyes of Lycosa punctulata was performed. A layer of pigment cells was found around the rhomboids of the posterior lateral eyes, posterior medial eyes, and the anterior lateral eyes, but not around the rhomboids of the anterior medial eyes (AME). The same condition exists for the family Salticidae. Each rhomboid has two rhodoneone faces associated with it, including the rhomboid of the AME. This condition differs from that described for the Salticidae in which each rhomboid of the AME has five rhodoneone faces associated with it. The rhodoneone faces are made up of microvilli like structures produced by evaginations of the cell membrane. Along the inner faces of the rhodoneone, microvilli like structures produced by evaginations of the cell membrane. Along the inner faces of the rhodoneone are apparent, lending evidence that Lycosidae exhibit receptor membrane breakdown as has been described for two families of spiders, the Diopсид and the Salticidae. The microvilli of the rhodoneone show various orientations with regard to the path of incoming light, a condition which may be the basis for detection of polarized light.

510.18 NEPHRIDIAL NERVE CELLS OF THE LEECH ARE SENSITIVE TO EXTERNAL Cl- CONCENTRATION. R.L. Calabrese and A. Wenning. Dep. of Biology, Emory Univ., Atlanta, GA 30322 and Fakultat für Biologie, Universität Konstanz, D-7840, Konstanz, F.R.G.

Nephridial nerve cells (NNCs), putative sensory neurons in the leech, Hirudo medicinalis, have a peripheral soma and extensive dendritic processes that invade a nephridium (Wenning, A., and Cahill, M.A., Cell Tissue Res., 245:397, 1986). Leech blood is normally low in Cl- (organic acids provide anionic balance), but after feeding Cl- increases more than two-fold, while cation concentration (mainly Na+) increases only slightly (Wenning, A., et al., J. Comp. Physiol., 139:97, 1980). NNCs respond to changes in the external Cl- concentration of the blood and fluid surrounding the nephridium but not to changes in osmolality or Na+ concentration (Wenning, A., J. Exp. Biol., in press).

Using intracellular recording and switching single-electrode voltage clamp techniques on isolated NNCs with intact dendrites, we showed that low Cl- (37.6 mM) salines corresponding to normal blood Cl- levels depolarize NNCs (V_m = -36.7 ± 9.9 mV), while high Cl- (152.6 mM) salines corresponding to postfeeding blood Cl- levels hyperpolarize NNCs (V_m = -55.6 ± 8.0 mV). NNCs respond to changes in external Cl- when it is replaced by SO_4^-, ethionate, malate, or succinate, but not when it is replaced by Br-. Low Cl- salines cause a reversible conductance increase and inward current in NNCs. This inward current is not blocked in 0 mM Na+ saline or by 5 mM Ca^2+ and is not voltage sensitive over the range of -65 to -40 mV.


There is accumulating evidence that powerline frequency magnetic fields can affect a variety of physiological processes. The present experiment examined the effects of various durations (0.5, 2, 12, 48 or 120 h) of day- and night-time exposure to a 10 gauss (rms) 60-Hz magnetic field on post-exposure mortality in land snails (Cepaea nemoralis). These snails were injected with morphone or saline and tested for reaction to an aversive thermal stimulus as part of another study. Mortality levels were monitored over a 2 week period and were shown not to be differentially affected by the drug injection procedure. Mortality levels increased significantly (p<0.05) with increasing length of magnetic field exposure and night-time exposure resulted in greater mortality than day-time exposure (p<0.05). These results indicate that day-night rhythms are important in determining the magnitude of the magnetic field exposure effect on mortality in these snails. (Supported by NSERC.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
511.1 NEURAL BASIS OF HEAD MOVEMENTS EVOKED BY OPTOMOTOR STIMULI IN THE MOTH MANDUCA SEXTA. J. Seyan, B. Hao, N. Strausfeld. Max-Planck-Institut für Biolo. Wissenschaften, Jena. Germany. E-mail: J.Seyan@mpib-jena.mpg.de. Optomotor stimuli evoke head movements in flying and as well as in non-flying animals. Even in an intracellular recording situation the visually guided activity of neck motoneurons and muscles can be monitored. This enables a direct correlation between the responses of visual interneurons in the brain and the motor output.


Selective staining of receptors and interneurons reveals specific differences of organization within olfactory neuropils of different insects. In Calliphora and Manduca olfactory centers receive more than one topologically distinct type of receptor terminal and is supplied by several receptor axon bundles each associated with discrete axon bundle. These are reflected by the lateral and stratification of several uniquely identifiable projection neurons (PNs) accompanied by many types of local interneurons [Kanzaki R, Arbas E, Strausfeld NJ, and Hildebrand J.G. (1989) J. Comp Physiol A. in press]. In Periplaneta and the honeybee Apis, only one class of receptor terminal innervates any glomerulus. PN dendrites fill a glomerulus and discrete partitions within its neophil are not resolved. In Periplaneta and Apis glomerular output neurons (PNs) send axons to complex higher centers (muscle body), which comprise many thousands of interneurons contributing to a sophisticated and highly structured neuroarchitecture [Mobbs, P (1982) Phil Trans Roy Soc Lond 298:309-351]. By comparison, complex glomeruli in Diptera and Lepidoptera are associated with simple muscle bodies, consisting of a few hundred neurons contributing to a diffuse neuroarchitecture. The anatomy suggests two possible evolutionary lines in olfactory pathway organization. In one, cross-fiber interactions amongst afferent relays are elaborate in the larval antennae, whereas in Hymenoptera and Orthoptera such interactions may predominate in the mushroom bodies.


The antennal lobe of the brain of adult male Manduca sexta comprises a male-specific macroglomerular complex (MGC) and an array of about 60-600 µm in diameter and >100 µm thick. Presynaptic profiles in the MGC is the principal or exclusive site for primary synaptic processing of sensory information about the female moth's sex-pheromone blend (Matsumoto et al., 1989). The MGC comprises a macroglomerular complex (Campanotus & Hildebrand, unpublished observations; Strausfeld, 1989). The neuronal interactions in the MGC are complex, involving both excitatory and inhibitory synapses (Christensen and Pinto, 1987) and a large, homogeneous population of primary receiving neurons (Yoshinari et al., 1989).

We are currently analyzing the ultrastructure of the MGC. In reconstructions from serial sections we identify two main parts, a lobulated "cap" and a deeper "toroid", as well as smaller structures; both the toroid and the cap are >500-600 µm in diameter and >100 µm thick. Presynaptic profiles in the MGC are large, round dense-cored vesicles. In work in progress, we are examining the distribution in the different synaptic profiles within the subunits of the MGC and identifying the neurons that form each type. [Supported by NIH grants NS20041 and AI12035 and by a fellowship from JSPS, Japan.]


As part of our ongoing work on sensory processing in the insect CNS, we are investigating the central projections of sensory neurons in lepidopteran larvae. Kent and Hildebrand [Phil Trans R Soc Lond B 315:3-36 (1987)] described the central projections of sensory neuron in lepidopteran appendages of the larval sphinx moth Manduca sexta. Here, in a complementary study, we report findings from larvae of the pest noctuid moth Heliolithis sex and \textit{H. sex}. We cut the sensillum appendages and stained their axons with cobalt-hydroxy followed by silver-intensification, using standard techniques. The tissue was either viewed in whole-mount or embedded in Epon and sectioned.

The central projections of the sensory neurones of the cephalic appendages are very similar in the two \textit{Heliolithis} species. Several different neuropil regions in the brain and the subesophageal ganglion receive primary-afferent inputs. The larval antennal center (LAC) comprises the deutoeophagi (DE) and subesophageal ganglion (SEG) receive inputs from both the antenna and the maxilla. The trocollateral appendages receive direct monosynaptic input from tactile hairs from the antenna, labrum, head and cuticle, while sensory afferents from the stomatogastric (larval eyes) project solely to the optic neuropil in the brain. The stained preparations show that there is an overlap of projections from the antenna and the maxilla, suggesting that the chemosensory inputs from these two appendages are processed in the same neuropil regions in the LAC and SEG.

Our findings from the antenna are almost identical to those from Manduca. That the organization of primary sensory pathways is very similar in larvae of different moth families supports our use of Manduca as a model for studies of sensory processing in lepidopteran larvae. [Supported by USDA grant 87-CRCR-1-2362, NIH Postdoctoral Fellowship NS37900, and Monsanto Co.]


Directionally selective (DS) motion sensitive neurons carry out the non trivial computation of sequence-discrimination. We have recorded from H1, one of the identifiable, large field DS neurons of the fly lobula plate (rev. Hausen K. and Egelhaaf M., in "Facets of Vision" Eds STAVENGA D. G., Harde D.C.R., Springer, New York, 1989) upon precise stimulation of a single Elementary Motion Detector (E.M.D.). Two 1µm spots were presented to two neighboring photoreceptor cells in sequence, thereby "simulating" a monosynaptic input. The small field H1 responds with a transient but vigorous spike discharge, provided the sequence simulates motion in the preferred direction (Riehle A. and Franceschini N., Exp. Brain Res. 64: 391, 1986). This functional diagram of this neurosystem has highlighted a fine-grained network of facilitatory controls that operate in both directions – preferred and non-preferred (Franceschini N. et al. in "Facets of Vision" ibid., 1989). We have now combined various sequences of stimuli such as pulses and/or steps (of light and/or darkness) on the two photoreceptor cells and determined the dynamic properties of the various filters that make up the various branches of a single E.M.D. Of particular interest is the preprocessing of signals which makes use of a nonlinear high-pass filter having a rectifying stage. Moreover this kind of filter is present on two E.M.D.'s that act in parallel to detect the motion of the leading edge and trailing edge of an object, respectively.
TRANSMITTER GATING, OPPONICITY, AND FACILITATION IN ELEMENTARY VISUAL MOTION DETECTION.

H. Gligoric, N. Gaggero, and J. M. Barone Dept. of Elect. Eng., Univ. of Houston, Houston, TX 77204. Dept. of Elect. Eng., Université Laval, Ste-Foy, Quebec, Canada, G1K 7P4

Tuesday, 7:30 A.M. INVERTEBRATE SENSORY SYSTEMS II

It has been suggested that motion detection in invertebrates is similar to that in vertebrates. However, invertebrate motion detectors are frequently monopolar cells with large receptive fields. This study aimed to elucidate the mechanisms involved in the detection of motion in invertebrate eyes.

The first stage of processing involves the detection of motion in the retina. Monopolar cells in invertebrates similar to these in vertebrates have large receptive fields.

The second stage of processing involves the integration of these signals over larger fields. This occurs in the lamina of Drosophila.

The third stage involves the combination of these outputs in the medulla. The complex receptive field profiles of the medulla are used to divide the image into distinct regions.

Finally, the outputs are combined through complex receptive field profiles to feed motion sensitive cells of the lobula. Analysis and simulations of the model show that it is in good agreement with experimental data.
**511.13**

**THE PUTATIVE ROLE OF ACTIN IN THE INNER SPACE OF THE OPEN-TYPE OMMAFITIA IN THE COMPOUND EYE.**

M. S. F. J. D. M. Masters. Dept. of Zoology, Washington Univ., St. Louis, MO 63130.

The photoreceptor cell microvilli, the functionally unitary parts of cell periaxons, are separated from each other by an extracellular, inneromatalial space (IFS), which composition and significance to the visual function were studied. The prefixed (25 PaO. 25% GA, O.1-n phosph. buss. n), frozen sections of the compound eye of the blowfly were cut and stained by immuno-fluorescence techniques, incubating by antibodies of actin, actinin, and spectrin and actin, actinin for both EM and immunofluorescence studies.

The immunocytochemical analysis shows that actin and filamin (not tubulin) are components of the IOS and not present in microvilli structures. The actin is located just outside of the microvilli, whereas spectrin is present along the photoreceptor membrane inside IOS. We propose an actin based filiment network to regulate optical adjustment of the ommatidia. This hypothesis is supported by the observation of a deep pseudopupil deformation after treatment of ommafitia with 0.1 M dibutyryl-cAMP solution known to lead to a rearrangement of cytoskeletal actin.

**511.14**

**CORRELATION BETWEEN INCREASE IN VISUAL SENSITIVITY AND BIONUCLINESS FLASING ACTIVITY IN NIGHT-ACTIVE FIREFLIES.**

J. Mogdans and H.-U. Schnitzler Department of Zoology, University of Tuebingen, Morgenstelle 28, D-7400 Tuebingen, FRG.

Range resolution in echolocating bats refers to their ability to decide, whether an echo contains only one wavefront, reflected from a single surface, or two or more wavefronts from two or more closely spaced surfaces. We examined the echolocation behavior of bats in a range resolution task. Big Brown Bats (Eptesicus fuscus) were trained in a two alternative forced choice procedure to discriminate between electronically delayed versions of their echolocation calls similar to one wavefront echo ($S$) and two-wavefront echoes ($S^2$).

The ability of Eptesicus fuscus to discriminate a one-wavefront echo from two-wavefront echoes was limited to distinct internal time delays between the wavefronts of the double-wavefront echoes. Analysis of the two-wavefront echoes revealed periodic minima in the spectrum. Position and separation of the minima depend on the time delay between the two wavefronts. The occurrence of minima within the frequency range of the first harmonic in the echo of the bats' echolocation calls correlates with the bats' ability to discriminate a one-wavefront echo from two-wavefront echoes.

From this finding we conclude that for range resolution Eptesicus fuscus uses spectral information of their sonar echoes rather than information about the arrival time of different wavefronts. (Supported by the Deutsche Forschungsgemeinschaft PS 357.)

**512.1**

**TRADEOFF BETWEEN ECHO CROSSCORRELATION-PEAK DELAY AND TIME DELAY IN TARGET RANGING BY BIG BROWN BATS.**

M. Masters. Dept. of Zoology, Ohio State Univ., Columbus, OH 43210.

If asked to choose the nearer of two electronically simulated "phantom" targets in a two-alternative forced-choice (2AF) task, big brown bats (Eptesicus fuscus) perform well if the simulated echoes are models of their own calls (range discrimination threshold about 18° to 20° target distance of 80 cm), but do poorly if the same echoes, reversed in time, are used (threshold typically 20 times larger) (Masters and Jacobs, unpub.). Clearly, time-frequency structure of the echo is relevant to the bat, indicating that Eptesicus uses some type of matched-filter processing to determine echo arrival time. This finding is consistent with the idea that FM bats use crosscorrelation (pulse, filter, pulse compression) reception of echoes. Thus, a bat's estimate of echo delay (and, hence, of target range) will be affected not only by the travel time of the echo (travel delay), but also by the time of occurrence of the crosscorrelation peak (peak delay) of the echo. In other words, two echoes with the same travel delay (i.e., same actual range) but different peak delays should seem to be at different distances. To test this idea, I modified the low-frequency structure of echoes to give different peak delays and then, in a 2AF task, determined the tradeoff between travel delay and peak delay in the bat's estimate of target range. Preliminary results with two bats suggest that the peak delay determined by crosscorrelation gives a good approximation of the apparent echostructure induced range shift perceived by the bat, again consistent with the possibility that FM bats use something closely akin to crosscorrelation processing of echoes for target distance.

**512.2**

**BIG BROWN BATS USE SPECTRAL ECHO CURVES FOR RANGE RESOLUTION.**


Range resolution in echolocating bats refers to their ability to decide, whether an echo contains only one wavefront, reflected from a single surface, or two or more wavefronts from two or more closely spaced surfaces. We examined the echolocation behavior of bats in a range resolution task. Big Brown Bats (Eptesicus fuscus) were trained in a two alternative forced choice procedure to discriminate between electronically delayed versions of their echolocation calls similar to one wavefront echo ($S$) and two-wavefront echoes ($S^2$).

The ability of Eptesicus fuscus to discriminate a one-wavefront echo from two-wavefront echoes was limited to distinct internal time delays between the wavefronts of the double-wavefront echoes. Analysis of the two-wavefront echoes revealed periodic minima in the spectrum. Position and separation of the minima depend on the time delay between the two wavefronts. The occurrence of minima within the frequency range of the first harmonic in the echo of the bats' echolocation calls correlates with the bats' ability to discriminate a one-wavefront echo from two-wavefront echoes.

From this finding we conclude that for range resolution Eptesicus fuscus uses spectral information of their sonar echoes rather than information about the arrival time of different wavefronts. (Supported by the Deutsche Forschungsgemeinschaft PS 357.)

**512.3**

**FURTHER ADAPTATION OF FM-FM NEURONS OF BAT'S AUDITORY CORTEX FOR RANGING.**


The matched bat Pteronotus parnellii emits biosonar pulses consisting of 8 components: CF$_1$ and FM$_1$. FM-FM neurons are tuned to a particular delay of echo FM$_1$ ($\approx$2.3, or 4) from pulse FM$_1$ for processing target-range information. The FM$_1$ is suited not only for ranging, but also for target localization. If FM-FM neurons are directionally sensitive, however, this would greatly interfere with the encoding of range information. Electrophysiological studies indicate that the receptive fields of FM-FM neurons are large, covering the entire contralateral auditory field and medial half of the ipsilateral auditory field. FM-FM neurons are not suited for sound localization, and their best delays (best range) change little with changes in the direction of the echo source. The best azimuths of FM-FM neurons for echo FM$_1$, FM$_2$, and FM$_3$ are similar to those of peripheral neurons. However, the best azimuth for pulse FM$_4$ (2° front) is quite different from that of peripheral neurons (25° lateral). Since FM-FM neurons are conditioned by self-vocalized pulse FM$_4$ to process target-range information, their directional sensitivity to pulse FM$_4$ is an adaptation of these neurons for ranging. (Supported by PHS research grant NS17333.)

**512.4**

**MUSCIMOL APPLICATION TO THE BAT'S AUDITORY CORTEX DISRUPTS FINE FREQUENCY DISCRIMINATION OF BIOSONAR SIGNALS.**

H. Rigoramus, S. J. Gaspary, and N. Suga, Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The auditory cortex (AC) of the mustached bat, Pteronotus parnellii, has a highly specialized region, the DSCF area, which overrepresents the frequencies of Doppler-shifted echoes (about 61 kHz), and hence may play a role in the fine frequency discrimination necessary for detailed velocity measurements (Suga, 1984). We behaviorally tested this function of the DSCF area using reversible ablation with muscimol, a potent GABA antagonist (Hikosaka et al., 1985). Bats were trained on a discriminated shock avoidance task requiring a leg flexion response. The stimuli were trains of artificial pulse-echo pairs (tone bursts). For S+ the pulse and echo were the same frequency (e.g., 61.0 kHz), while for S- they were different frequencies (e.g., 61.0 kHz and 61.1 kHz). Following baseline behavioral training, we applied muscimol/µl saline bilaterally to the DSCF area. The bats failed on discriminations of large frequency differences (e.g., 50 Hz), but succeeded on discriminations of small frequency differences (e.g., 2 kHz). Discrimination performance returned to baseline levels. The finding that the DSCF area is necessary for fine frequency discriminations involving biosonar signals. (Supported by AFOSR grant 87-0250.)
512.5
DELAY LINES IN THE INFERIOR COLICULUS OF THE MUSTACHEBAT. T. Hatton* and N. Suga (SPON: Y. Fukamizu). Dept. of Biology, Washington Univ., St. Louis, MO 63130
To process target range information, the auditory system of the mustached bat, Pteronotus parnellii, creates arrays of FM-FM neurons which act as delay-dependent multiplexers. The delay lines utilized by these neurons are as long as 18 ms, which is longer than the latency of the head, ears and body. (Supported by PHS grant NS17333)

512.6
The auditory cortex (AC) of the mustached bat, Pteronotus parnellii, consists of multiple functional areas that process specific types of biosonar information. Targets of several of these areas were identified by combining physiological mapping with anterograde and retrograde tract-tracing. The FM-FM area (a target range processing area) had the most extensive projections; labeled areas included:

Anterior J/C Subcortical Thalamus Midbrain cortex
1. Frontal 1. DF 1. Claustrom 1. MG 1. DLMT
2. Ant. Cing. 2. VF 2. Striatum 2. Other 2. SC
3. VM 3. Amygdala nuclei 3. PNM
4. IC
where DF, VF, and VA are distinct functional areas in the AC, MG=medial geniculate, DLMT=dorsolateral midbrain tegmentum, SC=superior colliculus, PNM=pontine motor nuclei, and IC=inferior colliculus.
Projections of the primary AC, the CF/CF area (a velocity processing area), DF and VF areas (additional range processing areas) were also identified, and included most of the structures identified above. The auditory cortex therefore contributes to multiple pathways involved in auditory sensory processing, vocalization control, and orientation of the head, ears and body. (Supported by PHS grants NS17333 and NS07057)

512.7
The FM-FM area of the mustached bat's auditory cortex consists of an array of neurons which respond to biosonar pulse-echo pairs with specific echo delays, forming a map of target range. Facilitation occurs when pulse and echo responses coincide, so that neural delays must vary systematically from short to long along a ventrolateral-dorsomedial axis, i.e., along the lateral lemniscal fibers terminating within the inferior colliculus. The latency axis from 4 to 12 ms (a delay line of 8 ms) occupies about 2 mm distance across the anterolateral division. Since the diameter of myelinated lemniscal fibers in the inferior colliculus is about 1 μm, the conduction velocity of action potentials is expected to be about 6 m/sec, and the conduction time over 2 mm is calculated to be 0.53 ms. Therefore delay lines are predominantly due to synaptic delays and/or phasic on-inhibition. (Work supported by PHS research grant NS17333)

512.8
For echolocation, the big brown bat, Eptesicus fuscus, emits orientation sounds consisting of two or three FM harmonics. Delay-tuned neurons in the intercollicular nucleus of this bat show a facilitative response to a synthesized pulse-echo pair, each containing the remaining three harmonics (Feng et al, Science, 202:645-648, 1978). Our focus is to identify the essential elements for facilitation using single FM harmonics. In unanesthetized bats, responses to pairs of linear FM sounds or pure tones were recorded using carbon-fiber microelectrodes. Best delays of delay-tuned neurons ranged from 0.5 ms to 26 ms and were systematically distributed from rostral (short delay) to caudal (long delay). Four-fifths of these neurons responded selectively to single harmonic pairs (FM1-FM2, etc) and one-fifth to multimodal pairs (FM1,FM2,FM3 etc). There was a high correlation between facilitation latency and best delay, r=0.96; but a poor correlation between pulse or echo alone latency and best delay, r=0.96 and r=0.47, respectively, suggesting that delay lines may not be the mechanism creating delay-tuned neurons in this bat. In order to identify the region containing delay tuned neurons, HRP was injected in several animals. The injection site was always located dorsal and caudal to the medial geniculate body, and ventral and lateral to the superior colliculus. Hence, we use the descriptive term: dorsolateral midbrain tegmentum. This region is located lateral and caudal to the intercollicular nucleus (Roberts et al., J Comp. Neurol. 170:499-526, 1978).
(Work supported by PHS grant NS17333 and PHS grant NS07057)

512.9
PHARMACOLOGICAL DIFFERENTIATION OF CONTACT CALLS IN ADULT MALE SQUIRREL MONKEYS. J. C. Harris and D. J. Newman. Lab. of Comparative Ethology, NICHD, NIH, Bethesda, MD, 20892.
The isolation peep and twitter are acoustically distinct vocalizations of the squirrel monkey (Saimiri). They are grouped into the same functional category, “contact calls,” due to their association with the establishment of social contacts between familiar conspecifics. Peripheral administration of appropriate doses of the opiate receptor antagonist, naloxone, and the alpha2-adrenoreceptor antagonist, yohimbine both increase production of the isolation peep in adult males visually and acoustically isolated from conspecifics (Harris and Newman, 1988). The combined administration of these 2 drugs increases production of the peep under the same testing conditions (Harris and Newman, 1988). To further understand the neurochemical regulation of these vocalizations, we performed additional analyses on the relationships between drug-related changes in production of these 2 call-types and the alpha2 receptors and the GABAA receptor. We determined the best delay as well as for sharpening delay tuning, GABA agonists and bicuculline methiodide (BMI) were iontophoretically applied to FM-FM neurons in both cortex and thalamus while recording from single neurons with multibarreled carbon-fiber electrodes. In over half of cortical FM-FM neurons, BMI increased the width of the delay tuning curve while GABA sharpened this curve. A high correlation between facilitation latency and best delay, r=0.96, but a poor correlation between pulse or echo alone latency and best delay, r=0.96 and r=0.47, respectively, suggesting that delay lines may not be the mechanism creating delay-tuned neurons in this bat. In order to identify the region containing delay tuned neurons, HRP was injected in several animals. The injection site was always located dorsal and caudal to the medial geniculate body, and ventral and lateral to the superior colliculus. Hence, we use the descriptive term: dorsolateral midbrain tegmentum. This region is located lateral and caudal to the intercollicular nucleus (Roberts et al., J Comp. Neurol. 170:499-526, 1978).
(Work supported by PHS grant NS17333 and PHS grant NS07057)

512.10
TYPE A AND B MONOAMINE OXIDASE INHIBITORS HAVE DIFFERENTIAL EFFECTS ON THE VOCAL AND MOTOR BEHAVIOR OF SOCIALLY SEPARATED SQUIRREL MONKEYS. J. D. Newman, J. L. Winslow, and D. L. Murphy. Lab. of Clinical Science, NIMH, and Lab. of Comparative Ethology, NICHD, NIH, NIHAC, Poolesville, MD, 20837.
Squirrel monkeys (Saimiri sciureus) were separated from familiar conspecifics for 10 days, when separated from familiar conspecifics. Previous work has demonstrated that primate vocalization is a sensitive measure of emotional state and may provide a potential tool for examining the brain mechanisms controlling emotional behavior. We studied the acute effects of monamine oxidase inhibitors L-deprenyl (1.0-5.0 mg/kg), clorgyline (1.0-5.0 mg/kg), and milnacipran (100-400 mg/kg) on the behavior of male squirrel monkeys during brief (15 min) social separations beginning 60 min after subcutaneous drug administration. Monkeys were tested once per week using a standard baseline calling rate, typically a minimum interval of 14 days between drug tests. All drugs were administered intramuscularly and the frequency and duration of an exhaustive catalogue of other behaviors. All three drugs selectively reduced the rate of calling during social separation at doses which did not alter time spent locomoting, nor the frequency of vigilance-checking. Deprenyl and milnacipran, but not clorgyline, produced concurrent decreases in locomotion at the higher doses tested. The data demonstrate the utility of the social separation paradigm for the evaluation of drug effects. The data also suggest that MAO-A inhibitors may selectively affect vocal behavior, while MAO-B inhibitors may be more generally involved in arousal. This is consistent with previous reports of noradrenergic and serotoninergic mediation of separation distress in rodents and non-human primates.
Development of tactile righting reflexes in the marsupial Dasyurus hallucatus. S.M. Pellis, W.P. Pellis and J.H. Nelson, Department of Psychology, University of Florida, Gainesville, FL 32611. During fetal development in the cat, tactile righting reflexes precede vestibular righting reflexes (Windle, W.P. and Fish, M.W., J. Comp. Neurol. 54: 85-96, 1932). However, the "tactile system" as it pertains to righting behavior, is not one, but three distinct systems. In this study, the order of appearance and maturation of these tactile righting systems are described and analyzed for the pouch young of a carnivorous marsupial. The first form to appear was trigeminal righting, in which tactile input on the face can trigger rotation to prone. Next to appear was asymmetrical body contact triggering righting of the hindquarters, and last to appear was asymmetrical body contact triggering righting of the forequarters. The first form of righting appears at about 40 days postnatally, with all three maturing to the adult form by about 60 days.

EIGHT ARM RADIAL MAZE PERFORMANCE AND SOME RELATED BEHAVIORAL CHARACTERISTICS OF THE MEADOW VOLE, Microtus pennsylvanicus. Maureen M. Murphy, T.H. Ossenkop, N.K. Innis and F.H. Boon. Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 3C1. Eight arm radial maze performance was assessed in common meadow voles, Microtus pennsylvanicus. Based on maze performance and behavior patterns, voles were grouped into three categories: non-runners, strict-algorithmic runners and non-algorithmic runners. Some additional behaviors examined were performance complexity and spatiality, on a passive avoidance task, and spontaneous locomotor behavior in an automated Digiscan apparatus. Relative to non-runners, strict-algorithmic runners were more active on measures of horizontal and vertical activity, and average speed and number of movements. On the passive avoidance task, non-runner voles showed much slower extinction of the task than did the other two groups. These results indicate that differences in eight arm radial maze performance in voles are a function of certain characteristics of spontaneous locomotor activity and are also reflected in performance on other tasks (Supported by NSERC).

EARLY LESIONS PRODUCE DISSOCIATIONS OF THE ANTI-PREDATOR AND ORIENTING FUNCTIONS OF SUPERIOR COLLICULUS S.L.Ayres. Development of tactile righting reflexes in the marsupial Dasyurus hallucatus. S.M. Pellis, W.P. Pellis and J.H. Nelson, Department of Psychology, University of Florida, Gainesville, FL 32611. During fetal development in the cat, tactile righting reflexes precede vestibular righting reflexes (Windle, W.P. and Fish, M.W., J. Comp. Neurol. 54: 85-96, 1932). However, the "tactile system" as it pertains to righting behavior, is not one, but three distinct systems. In this study, the order of appearance and maturation of these tactile righting systems are described and analyzed for the pouch young of a carnivorous marsupial. The first form to appear was trigeminal righting, in which tactile input on the face can trigger rotation to prone. Next to appear was asymmetrical body contact triggering righting of the hindquarters, and last to appear was asymmetrical body contact triggering righting of the forequarters. The first form of righting appears at about 40 days postnatally, with all three maturing to the adult form by about 60 days.

THE COMPARATIVE NEUROBIOLOGY OF AFFILIATIVE BEHAVIOR AND SOCIAL BONDING: PRAIRIE AND MONTANE VOLES AS A MODEL SYSTEM. L. SHAPIRO and C. MEAD. Laboratory of Comparative Neurobiology, Department of Zoology, University of California, Berkeley, CA 94720. The comparative, neurobehavioral analysis of interspecies differences exemplifies a useful approach for investigating fundamental brain-behavioral relationships. Prairie and montane voles are dealt for about closely related phylogenetically and morphologically. These two species display marked differences in social organization in the wild. Field data indicate that prairie voles are monogamous and live in natal monogamous. Montane voles are polygamous, solitary, and females abandon their young at about 2 weeks after birth. Laboratory studies have also revealed differences in such affiliative processes as partner preference, pair bonding, and parental behavior (e.g., Shapira et al., in press). The aim of the present research is to identify the neural mechanisms underlying these interspecies differences in affiliative behavior.

Foraging rats display different motor behaviors depending upon the size of pellets of food that they encounter. Small food pellets (20-45 mg) are swallowed, medium food pellets (74-190 mg) are held in the forepaws to be eaten, and large food pellets (>300 mg) are carried (hoarded) to available enclosures. The motor patterns associated with foraging also vary: when approaching food the rats use a stalking walk or trot, interrupted with pauses, but when carrying food they gallop. If hoarding is frustrated by blocking the home cage entrance, motor behavior is still influenced by food pellet size: the rats dodge the food source with the pellet before eating it and dodge distance increases with food pellet size. The various motor behaviors associated with foraging can be differentially modified by environmental change as well as by central nervous system manipulations.

For example, swallowing and eating are influenced by the size context in which a food pellet is given but hoarding is not. Frontal and amygdala damage does not affect swallowing and eating but after the former damage food is treated as being smaller than it is while after the latter damage food is treated as being larger than it is. Hippocampal damage abolishes hoarding. The motor patterns of hoarding can also be differentially influenced by pharmacological manipulation, i.e., anticholinergics or monoaminergic drugs. Thus, the motor patterns of hoarding provide a rich selection of movements with which to analyze sensory influences and neural control of foraging.


Anatomy: In a series of careful mapping studies we have observed that every part of the hypothalamus may be unique and that the organization of (1) thalamicoutletarchitecture, (2) meyoarchitectures, (3) localization of various (neuro_medianator-specified) components of the Medial Forebrain Bundle, and (4) the destination of its efferent fibers. Many of these fibers descend into the brainstem via the tractus "paracere" (Nieuwenhuijse et al., '85, '89), and may play a prominent role in the natural or artificial elicitation of behaviour.

Behaviour: Specific behavioural responses can be elicited by electrical stimulation of different but partially overlapping parts of the hypothalamus in the rat (Lamers et al., '87, '88b). Self-grooming can also be induced by microinjection of kainic acid near the paraventricular hypothalamic nucleus (Roeling et al., in prep.). A detailed study of this and other "behaviorally defined" parts of the hypothalamus is now performed in order to specify the differences between the different "eering" of the neural substrate for grooming, aggression and possibly other kinds of behaviour.

BEHAVIORAL IMPORTANCE OF THREE "L"-SHAPED AUDITORY INTERNEURONS WHICH CO-EXIST IN THE PROTHORACIC GANGLION OF THE CRICKET ACHETA DOMESTICA. G. Atkins, S. Atkins and J. Stout. Dept. of Biology, University of Manitoba, Winnipeg, Manitoba, Canada.

The morphology of three auditory interneurons is described for Acheta domestica. Although L1, L2 and L3 share a similar gross structure there are differences which can be identified each of the neurons and which are always correlated with certain response properties. The coexistence of L1, L2 and L3 is confirmed by staining all three in a single hemiganglion.

In preparing a technique for evaluating the behavioral role of identified auditory interneurons in the cricket, individual neurons were photocautivated with blue light after being filled with Lucifer Yellow. Orientation preference and sensitivity to stimulus parameters and the response in the behavior were attributed to elimination of the killed neuron. We have repeated these unilateral experiments using a non-compensating treatment for the behavioral effects which allows for control by preliminary experiments for longer periods of time and have duplicated and extended our earlier results. Of particular interest, killing an ON1 results in angular deviations of 5-10° at the threshold of L3 but well above the threshold of ON1).

To test the necessity of a mirror-image pair of interneurons one would have to photoinactivate both members in the same animal. Since there are three pairs of "L"-shaped interneurons which are closely associated with each other in the anterior ring tract, the chances of a bilateral kill of the left and right member of one type is small. To avoid this problem we have begun to examine phonotaxis in "one-eared" (one tympanum waxed) crickets. Unilateral killing of a neuron on the intact side of a "one-eared" cricket allows us to evaluate phonotaxis with both members of a given pair inactive.


Vestibular dysfuction was induced in Long-Evans rats by intratympanic injections of sodium arsenate (arsonyl) following a 1 week recovery period the rats were tested for labyrinthine integrity by examining the loss of the air-righting reflex and the righting reflex (by lightly touching the back of the body at the sides against the wall) and the animals were then given two 10 min open-field tests in which odour detection, testing, grooming and defecation responses were recorded. Two weeks after all rats were tested twice in the automated Digiscan apparatus for 30 minutes.

Rats with vestibular dysfunction exhibited significantly more open-field ambulation but fewer rearing responses (p<0.01). In the Digiscan apparatus the arsenyl injected rats displayed significantly less time rearing (p<0.01), although number of vertical movements did not differ significantly from the control rats. Time per vertical movement was greatly reduced in the experimental rats (p<0.001). (Supported by NSERC.)

EFFECT OF STIMULUS INTENSITY ON DISCRIMINATION OF ODORANT MIXTURES BY SPINY LOBSTERS IN AN ASSOCIATIVE LEARNING PARADIGM. J.B. Fine-Levy and C.D. Derby. Dept. of Biology, Georgia State University, Atlanta, GA 30303.

Previously, we have shown that the Florida spiny lobster, Panulirus argus, can behaviorally discriminate between members of four artificial odorant mixtures: crab, oyster, mullet, and shrimp. In our previous paradigm, each group of lobsters tested was conditioned to two concentrations (0.05 and 0.5 mM) of the conditioned stimulus in order that they attend to stimulus type rather than concentration. They were then tested with the same two concentrations of each of the non-conditioned stimuli. In this present experiment, we designed to examine the effects of stimulus intensity on inter- as well as intra-type mixture discrimination. This was accomplished by conditioning lobsters to only one concentration (0.5 mM) of shrimp mixture and testing them with three concentrations (0.005, 0.05, and 5.0 mM) of shrimp mixture and two concentrations (0.05 and 0.5 mM) of shrimp mixture. Preliminary data suggest that lobsters conditioned in the present paradigm continue to show a robust discrimination of oyster mixture from the 0.5 mM shrimp mixture, as well as an impressive recognition of oyster mixture versus the nonconditioned shrimp mixture concentrations. These inter-type differences are significantly larger than the observed intra-type differences. (Supported by NINCDS Grant No. NS22225 and a Whittall Foundation Grant.)


Before jumping to a target locusts induce motion parallax by side to side "peering" movements (Wallace, G. K., J. Exp. Biol. 36:512-525, 1958; Collett, T. S. J. Exp. Biol, 76:237-241, 1978). We presented 4th and 5th instar locusts (Schistocerca americana) with vertical black rectangular targets at varying distances (subtending constant visual angle) which they jumped to when direct heat on their perch increased slowly. Jump velocity increased as target distance increased suggesting that locusts have distance perception.

By electronically tracking the head position of the animal while it peered at a target, the target could be moved laterally during peers to create the proximal stimulus of a target closer or farther from the locating locust. We found that the artificial parallax and jumped with the same velocity toward targets at an illusory distance as they did to targets actually at that distance, demonstrating distance detection by motion parallax.

Locusts are sensitive to the absolute value but not the sign (direction) of motion parallax. When presented with paradoxical parallax (a target whose motion parallax describes a negative distance from the animal i.e. behind the animal) locusts jump forward with the same velocity as if the target were located at the absolute value of the computed distance. Supported by M17168
512.23

THORACIC INTERNEURONS OF THE VENTRAL GIANT SYSTEM EXCITE LEG MOTOR NEURONS OF MULTIPLE GANGLIA DIRECTLY AND VIA LOCAL INTERNEURONS. A.J. Pullukat and R.E. Pfeiffer. Dept of Biology, Case Western Reserve University, Cleveland, OH 44106.

Our laboratory has been studying the wind mediated escape system of the cockroach Periplaneta americana as a model which will allow us to determine how populations of interneurons interact to control orientation behaviors. As part of this long term investigation, we have identified a large population of thoracic interneurons (type A TIs) which are excited by ventral giant interneurons (vGIs) over constant short latency connections.

Since this population of TIs is the point where sensory information from vGIs is integrated and presumably utilized to control motor activity, we wanted to examine the pathway from type A TIs to motor neurons both in the ganglion in which the TI originates and in distant ganglia. We used paired intracellular recording and dye injection techniques to map the motor pathway. Our data demonstrate that both leg motor neurons and local interneurons are excited by type A TIs over constant short latency connections. Thus, the motor neurons are activated by these TIs both directly and by an indirect pathway that may serve to better coordinate multiple motor activities. Connections were demonstrated both in the ganglion of origin for the TIs and in distant ganglia. To date all connections between TIs and motor neurons or local interneurons in more anterior ganglia have been excitatory, whereas all connections in more posterior ganglia have been inhibitory.

This work was supported by NIH grant NS 17411 to R.E.R.

513.1

THORACIC INTERNEURONS OF THE VENTRAL GIANT SYSTEM EXCITE LEG MOTOR NEURONS OF MULTIPLE GANGLIA DIRECTLY AND VIA LOCAL INTERNEURONS. A.J. Pullukat and R.E. Pfeiffer. Dept of Biology, Case Western Reserve University, Cleveland, OH 44106.

Our laboratory has been studying the wind mediated escape system of the cockroach Periplaneta americana as a model which will allow us to determine how populations of interneurons interact to control orientation behaviors. As part of this long term investigation, we have identified a large population of thoracic interneurons (type A TIs) which are excited by ventral giant interneurons (vGIs) over constant short latency connections.

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This work was supported by NIH grant NS 17411 to R.E.R.

513.2


Local interneurones process mechanosensory signals from the cercal hairs of a locust and convert it into appropriate reflex movements that adjust posture and locomotion. The first stage in this processing is performed by spiking local interneurones which receive short latency inputs from exteroceptive afferents and make inhibitory synapses with other spiking and nonspiking local interneurones, with intersegmental interneurones and with some motor neurones. Each interneurone is excited by a particular array of receptors that form part of its excitatory receptive field. Two questions about the organisation of these fields are posed. First, how effective is a sensory spike in evoking a spike in an interneurone? Second, are all receptors within a field equally effective? These questions have been answered by recording intracellularly from an interneurone and monitoring the spikes of many afferents in its receptive field. There is a gradient of effectiveness within a field. An afferent from a hair toward the edge produces small EPSPs that are unable to make the interneurone spike. Hairs in the centre of a field may, by contrast, evoke large EPSPs so that each afferent spike reliably evokes a spike in the interneurone. Supported by NIH grant NS18008 and by SERC (UK).

513.3


We have examined the morphology of CoS silver-intensified leg and wing motor neurons in the metathoracic ganglion of the grasshopper Schistocerca americana, in whole mounts and reconstructed serial sections. The motor neurons fall into stereotyped and invariant anatomical groups in the cortex of the ganglion. Within a group the cell bodies are clustered and the primary neurites enter the neuropil at the same point, forming a discrete bundle. The bundles are distinct from one another and can be reidentified in different individuals. Primary neurite bundles are separate from bundles of motor axons arising from sensory axons entering the neuropil. The groups of neuronal cell bodies appear to be separated from each other by invaginations of glial cytoplasm.

The motor neurons within an individual group contribute axons to 1-3 peripheral nerves, and supply from 3-7 muscles. The motor neurons of a single muscle may fall into one group or separate groups. They do not appear to be mapped topographically by peripheral nerve or muscle innervated, or functionally by their involvement in a particular behavior. Instead, we suggest that the cortical organization of motor neurons reflects events early in the development of the nervous system.

513.4


We are interested in discovering the constraints on diversity of functional and morphological properties in the progeny of single neuroblasts. The DUM neurons in the grasshopper are the progeny of the single unpaired median neuroblast (Goodman & Spitzer. Nature 280:208, 1979). In the adult the DUM neuron group contains some 20 larger cell bodies (diameter 50-80um) roughly anterior to some 60-70 smaller cell bodies (15-25um). The large DUM neurons are octopaminergic afferent modulatory neurons (Evans & O'Shea. JEB 73:235, 1978). Only these larger neurons stain with Aq-S, consistent with the involvement of copper in octopaminergic biosynthesis (Wallace. J. Neurochem 26:761, 1976). In contrast, the small DUM neurons, but not the large ones, express GABA-like immunoactivity suggesting that they may function as inhibitory neurons.

Intracellular electrophysiological and morphological techniques were used to investigate the members of the small DUM neuron group. We have found them to be broadly divided into two classes: 1) local auditory interneurones, 2) intersegmental interneurones with diffuse branching patterns, that fire in association with voluntary movements of the animal. Both types have passively-conducted soma spikes (5-15mV) and display a variety of shapes, but are bilaterally symmetrical. Most project to the mesothoracic ganglion, or branch locally in the auditory neuropil.
TEMPERATURE EFFECTS ON LOCUST FLIGHT. J.A. Foster* and R.M. Robertson (SPON: L.Z. Wise). Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

Thoracic temperature in flying locusts can be greater than 35°C but the effect of such temperatures on the properties and interactions of flight interneurons is unknown. This study characterized the relationship between thoracic temperature and wingbeat frequency in intact, tethered Locusta migratoria. Local application of a wind tunnel for 1–3 hours while monitoring thoracic temperature and wingbeat frequency. At an ambient temperature of 24°C, the start-up stage was characterized by an increase in both thoracic temperature and wingbeat frequency. At the transition from start-up to steady state flight, there was a gradual decrease in thoracic temperature and wingbeat frequency to a level which was maintained for up to two hours. Artificial elevation of ambient temperature led to a decrease in thoracic temperature and a corresponding increase in wingbeat frequency.

MECHANISMS OF DEPOLARIZING SYNAPTIC INHIBITION AT THE GIANT MOTOR SYMPLAE OF CRAYFISH. D.H. Edwards, Biology Department, Georgia State University, Atlanta, GA 30303.

The crayfish giant motoneuron (MoG) is excited by giant interneurons through the electrically rectifying giant motor synapses and inhibited by depolarizing IPSPs from other interneurons. I have found three novel inhibitory mechanisms which activate both pre- and postsynaptic depolarization that supplement the previously described GABA-mediated increase in membrane conductance. (i) depolarization reverse-biases the rectifying electrical synapse and so decreases excitatory synaptic current through it; (ii) decreases the input resistance of MoG and so adds to the increase in postsynaptic membrane conductance, and (iii) it inactivates volleys of spontaneous synaptic action potentials; and (iv) so raises the motoneuron firing threshold. All four mechanisms produce similar degrees of inhibition. Mechanism (i) is likely to operate at other rectifying electrical synapses in crayfish and elsewhere. Mechanisms (ii) and (iii) may operate at depolarizing inhibitory synapses in vertebrates and invertebrates, including those that produce primary afferent depolarization. Supported by NIN Research Grant NS26457.


A population of flexion producing interneurons (F3FPs) bilaterally excite flexor motoneuron E3. Since mechanical stimulation of the swimmer inhibits the F3FPs and E3, we tested the possible GABAergic nature of this inhibition. Bath application of 5 µM picrotoxin produced a mixed response to swimmeret stimulation of IPSPs intermingled with a few spikes in both neurons. Amplitudes of their sensory evoked and spontaneous potentials were nearly abolished. Both GABA levels (20µM) further reduced sensory evoked response while increasing the spiking response. At higher concentrations (100µM), sensory evoked and spontaneous IPSPs were completely blocked, with F3FP and E3 responding to swimmeret stimulation with a burst of spikes. Picrotoxin effects of picrotoxin were not reversible. Bath application of GABA (100µM-500µM) decreased amplitudes of swimmeret evoked IPSPs, and spontaneous IPSPs and IPSPs by as much as 50% in F3FP and E3. Nicopotic acid (500µM) enhanced the frequency of IPSPs in both cells. These results indicate that the inhibitory mechanism of F3 FP and E3 is produced by GABAergic interneurons.

PRE- AND POSTSYNAPTIC MECHANISMS OF GABAERGIC INHIBITION AT A CRAYFISH SENSORDMOTOR SYMPALAE. B.M. Rawat* and P. Shungupk (SPON: B.Mathews). Dept. of Physiology, University of Bristol, Bristol, UK.

A non-spiking primary afferent receptor (the T-fibre) from the base of a crayfish leg makes direct monosynaptic excitation and disynaptic inhibitory connections with promotor motoneurones. The reflex expression of these alternative pathways is correlated with the phase of centrally generated motor activity and regulated by both pre- and postsynaptic factors. One factor may be GABAergic inhibition. GABA is an inhibitory neurotransmitter in the CNS of both vertebrates and invertebrates. In its actions are known to be modulated by two receptor subtypes, GABA-A and GABA-B. GABA-A receptors are coupled to a Cl- channel and predominantly located postsynaptically. GABA-B receptors are not coupled to a Cl- channel and are often found postsynaptically (although both subtypes have been found pre- and postsynaptically).

Bath application of GABA to the isolated crayfish thoracic nervous system has both pre- and postsynaptic effects. GABA (at 10-4 M) depolarises the central terminals of the T-fibre with an increase in conductance. This effect can be reversed when the T-fibre is held depolarised at 10-15MV from rest. GABA (again at 10-4 M) hyperpolarises promotor MNs, though with a smaller rise in conductance. Both effects are reversible. Baclofen, a GABA-A agonists, (at 10-5 M) does not alter the membrane potential or input resistance of the T-fibre, but does hyperpolarise promotor MNs, though with a smaller rise in conductance. This effect is reversible and persists in the presence of the GABA-A antagonist picrotoxin (at 10-5 M).

These preliminary results suggest that in this crustacean system both classes of GABA receptor are present, with a different pre- and postsynaptic distribution. This system, which has the advantage of being accessible, may be a useful model for the study of central GABAergic inhibition.

Supported by a SERC grant to B.M.Bush. B.M.Rawat is a SERC Scholar.

A NEUROANATOMICAL STUDY OF SEGMENTAL INTERNEURONES IN THE LARVAL AND ADULT STAGES OF THE INSECT, MANOCUL SEXTA. T.M. Amos* and K.A. Mesce. Graduate Program in Neuroscience and Department of Entomology, Univ. of Minnesota, St. Paul, MN 55108.

The holometabolous insect, Manocula sexta, has been the focus of much research regarding the sensory input underlying various behaviors; much less is known about the identities and behavioral roles played by interneurons. Many rhythmic motor patterns, including walking and ecysis (the shedding of the old cuticle), are thought to involve intersegmental interneurons that assist in the coordination of neuronal networks residing in the abdominal and thoracic ganglia. Segmental interneurons may also have a neurodevelopmental function, for example, pheromorphan interneurons determine whether the adult will deposit pheromones like ecysis behavior. Even the programmed death of particular abdominal motoneurons is influenced by putative intersegmental neurons descending from the pheromorphan ganglia.

To determine the numbers and locations of intersegmental interneurons present in the larva and adult, we constructed neuroanatomical maps of those neurons whose cell bodies originated in a given ganglion and whose processes (both ascending and descending) spanned multiple ganglia. Cobalt-bromide (1-3%) or cobaltium-lxine (<100mM) was applied to cut ends of ganglionic connectives at various anterior and posterior levels to stain the neurons. In the larva, sets of morphologically homologous neurons were found whose axons traveled through nine or more ganglia. In both the larva and adult, the numbers of intersegmental neurons per ganglion were found to be the same as or no more than half of the neurons found in regions relatively large (40um) although quite small somata (<15um) were also observed. Maps of these neurons will be valuable in determining whether postembryonic neurogenesis plays any role in the production of the adult intersegmental interneurons.

WHITE NOISE ANALYSIS OF REFLEX STIFFNESS IN HERMIT CRAB ABDOMINAL MUSCLE. William D. Chappell, Dept. of Physiology and Neurobiology, University of Connecticut, Storrs CT 06269.

Previous measurements (Chappell 1985) of the increase in muscle stiffness produced by a stretch reflex in the abdominal ventral superflicial muscle of the hermit crab, Pagurus pollicarius, using ramp stretches of the muscle indicated the reflex produced only a modest increase in muscle stiffness. The present experiments were designed to confirm these results and to characterize the action of the stretch reflex in the frequency domain. Bandpass Gaussian noise with a maximum amplitude of 6% of muscle optimum length was used to perturb the muscle while force and medial motoneuron frequency were recorded. Fast Fourier transforms of length (input) and force (output) were used to calculate the frequency response spectral density function and the coherence noise sequence under three conditions: 1) entire abdominal nerve cord, 2) 4th ganglion alone, and 3) passive muscle. Both 1 and 2 had stiffness magnitudes twice that of the control (3), but did not modify the dynamics of the muscle.
513.11

In crickets, movements of the antennae are controlled by 7 muscles. Cobalt backfills from these muscles revealed 18 motoneurones, of which one, with its cell body in the medio-ventral deutocerebrum, was filled from 6 of the 7 muscles. Thus, this motoneuron sends axon collaterals to several muscles, similar to common inhibitory (CI) neurones in the thoracic ganglia of insects.

Two lines of evidence support the idea that this antennal motoneuron is a CI. Firstly, electrical stimulation of one of these muscles innervated by this motoneuron caused a CNS artefact of the same latency and duration as that evoked by CI neurones in the thoracic ganglia. The second line of evidence is that when two or more antennal muscles were stimulated simultaneously, the contractions were additive. Thus, this motoneuron, which innervates the antennal muscles, is likely to be a CI neurone.

513.12

In order to understand the neural control of unsteady aerodynamics utilized in sophisticated flight, dragonflies have been studied. The interactions between sensory inputs and network properties that underlie the various flight modes are not well understood. To study the neural control algorithms responsible for the control of flight, extracellular multiple-unit recordings and single-unit analysis and correlation matrices, were performed in order to characterize the sensory, local, and global network control of flight. Changes were observed in the spatio-temporal interactions between groups of cells. Results indicated that variations occurred in the neural activity patterns and interactions both at the local, and global level. These changes were responsible for the control of various phases in the pre-flight to post-flight regime. The significance of these global control algorithms as they relate to the network and sensory control of flight is discussed.

513.13

To obtain more detailed information on the overall organization of the buccal ganglia and innervation of the buccal musculature, we have systematically backfilled each of the buccal nerves using CoCl2 and are mapping the motor projections of these nerves using extracellular and intracellular recordings. Backfills of all buccal nerves (N1-6) show both ipsilateral and contralateral cell bodies, although soma positions are unique for each nerve. The search for cell bodies, although soma positions are unique for each nerve. The search for sensory and/or motor functions for particular nerves, secretory and synaptic properties of particular nerves, revealed unique motor projections for nerves N1 and N4-6. One unique result was obtained from N3 (salivary nerve) backfills: most stained processes formed a single bundle which traveled near the ganglion and extended via N2. A few (1-5) of these processes separated from the bundle, entered the ipsilateral ganglion, crossed the commissure, and exited via contralateral N2. This result, combined with the fact that very few somata stained from N3, suggests that the salivary gland may be under substantial direct peripheral control from the gut. With these studies we can further define functional groupings of neurons in the buccal ganglia. Supported by NIH grant NS24262.

513.15

In Aplysia, swelling of the lips during food-induced arousal and phasic sculling of blood flow during feeding movements are probably mediated by the nervous system. The innervation of the posterior cardiovascular system is well understood. The motor innervation of the anterior arterial system has not been studied. We have recently shown that branches of the anterior aorta supplying the lips were rich in axons exhibiting yellow and blue glycylxyl isoleucine (PHI) or L11 peptide-like immunoreactivity.

Intracellular recordings from muscle fibers after POP cell firing reveals an increase in e.j.p. amplitude. POP cells contain serotonin-like immunoreactivity, and bath-applied serotonin (10 uM) mimics the POP cell effects. Supported by NIH, NS 27314, NS 07185 and NSF BBS 8711368.

513.16

The heart of Aplysia montorensensis is regulated by a small number of powerful motor neurons. We have identified five cardiac motor neurons in the CNS. Two motor neurons in the pleural ganglion, an excitatory and an inhibitory, have particularly potent actions on the heart. Low levels of spontaneous activity in either cell significantly alter the amplitude and rate of cardiomyocel contractions. These motor neurons in the visceral ganglion, one excitatory and two inhibitory, are less effective in altering cardiac activity, although they exhibit action potentials similar to their counterparts in the pleural ganglion.

We are currently investigating the activity of molluscan cardiac motor neurons in isolated pacemaker tissue of the Aplysia heart. AC decreases the amplitude, rate, and tension of heart contractions in a dose-dependent manner with a threshold of 10^{-6}. ACh, with a half maximal effect of 10^{-7}, excites the heart, predominantly increasing the amplitude of contraction. Two neuropeptides, small cardioactive peptide B and FMRFamide increase the amplitude and the rate of contraction with thresholds of ~10^{-6} and ~10^{-4}, respectively. Cardiac fractions isolated from the nervous system of A. montorensensis, sensitive and insensitive components. Supported by a K21 NR Training Grant of Research.
A VIDEO-MICROSCOPIC STUDY OF THE LEECH FEEDING APPARATUS DURING INGESTION. C. M. Lent and D. Zunde*. Department of Biology, Utah State University, Logan UT 84322.

The stereotyped ingestive behavior of Hirudo medicinalis is characterized by Lent, C., et al., J. Exp. Biol. 137:513, (1988). Protruded biting by jaws during ingestion has been suspected, but the opacity of flesh and blood has prevented confirmation. Leechees are now known to ingest a transparent fluid (1M Arg, 50M NaCl, Elliot, E. J. comp. Physiol. A 158: 381, 1986) upon which Hirudo imbibe in describing the activity of their three jaws during ingestion. Fluid (<37°C) was poured into a cylinder, leechees bit on a Paraffin™ membrane, and exhibited movements and durations typical of blood meal ingestion. Rhythmic biting movements not only cut into the film, but persisted throughout the 30 minute ingestive period. Jaw movements are synchronized to the pharyngeal rhythm. Both rates begin at ~3 Hz and decay logarithmically until termination. The depth of jaw insertion increases during ingestion and saliva accumulates visibly on jaw cutting edges. The power stroke of the biting movements appears to be the oral direction since its velocity exceeds the oral movement. Three to five seconds before the leech terminates ingestion, biting and pharyngeal movements cease completely. Supported by a grant (to C.M.L.) from the Willard L. Eccles Charitable Foundation.


A semi-intact preparation has been developed in which Intracellular recordings may be made from motoneurons (MS) while locomotory movements are in progress. Four classes of MS have been investigated: VI (ventral inhibitor), DI (dorsal excitatory), DE1 (dorsal excitatory, type 1), and DE2 (dorsal excitatory, type 2). The electrical activity in these neurons consists of slow potentials in VI and DI MS, ipps in DE1 and DI MS, and ipps in DE2 MS. These activities occur in bouts: DE1 ipps and VI slow potentials are in antiphase, and DI slow potentials are in antiphase with both the DI ipps and DE2 ipps. Simultaneous DE1 and DI ipps are in antiphase but are in phase with slow potentials in DI. This pattern of activity suggests that the neurons may be involved in the control of locomotory movements. The results of this study suggest that the neurons may play a role in the control of locomotory movements.

ION CHANNEL MODULATION AND REGULATION II

DEPHOSPHORYLATION OF RAT BRAIN SODIUM CHANNELS BY PURIFIED PHOSPHOPROTEIN PHOSPHATASES. S. Roussos* and W.A. Catrallin. (SPON: L. Halpern). Department of Pharmacology, University of Washington, Seattle, WA 98195.

The voltage-sensitive sodium channel purified from rat brain consists of subunits, α (290 kDa), β (36 kDa) and β2 (33 kDa). The channel-forming α subunit is phosphorylated by protein kinase A and protein kinase C. Phosphorylation of the channel by these enzymes increases its activity, leading to an increase in the number of sodium channels and an increase in the rate of sodium current. Dephosphorylation of the channel by protein phosphatase II leads to a decrease in the number of sodium channels and a decrease in the rate of sodium current. The effect of dephosphorylation on the number of sodium channels is mediated by a decrease in the number of phosphorylated subunits. The effect of dephosphorylation on the rate of sodium current is mediated by a decrease in the number of active sodium channels. The results of this study suggest that the dephosphorylation of sodium channels may be a regulatory mechanism that controls the activity of the sodium channel.
ATP AND ADENOSINE MODULATE CHANNEL ACTIVITY OF THE ACETYLCHOLINE RECEPTOR AT THE NEUROMUSCULAR JUNCTION.

F. W. Brown, R. C. Mackie, B. R. Buchanan, B. M. Moran. Department of Physiology, University of Wisconsin, Madison, WI 53706.

ATP and adenosine, released from motor nerve terminals, activate the ACh receptor. ATP and adenosine interact with the ACh receptor, but at different binding sites. A two-site model is consistent with the observations that 5'-AMP-PNP is a competitive antagonist of ATP and adenosine and that the dissociated adenosine metabolite, adenosine 3'-(N6)-phosphate (N6-salpinipside) is a modulator of ATP modulated AChR activity. ATP at concentrations of 10 μM in the pipette results in a 2-3 fold increase in surface AChR with a proportional increase in internal AChR. In contrast, ATP at micromolar concentrations of adenosine in the pipette, channel-opening probability induced by 0.1 μM ACh was increased by at least 30% compared to values obtained with ACh alone. Therefore, in addition to its inhibition of pre-synaptic ACh release, adenosine may also modulate ACh-receptor channel activity. Preliminary experiments indicate that a second messenger may be involved. Supported by NIH grant NS 13552 and the D.A.A.


Different stimulators of cAMP-dependent protein kinase (PKA) were used to investigate the regulation of expression of Torpedo nicoitonic acetylcholine receptors (AChRs) stably expressed in mouse fibroblast cell lines [Claudio et al. (1987) Science 238:1688]. As assayed by [3H]-bungarotoxin binding, an increase (1.5-3 fold) in surface AChR was detected after treatment with 3 different PKA stimulators: forskolin (10-100 mM), chlorophenylthio (CPT) cAMP (0.5-10 μM), and cholera toxin (100 μM). Forskolin treatment resulted in a 1.5-3 fold increase in surface AChR and a proportional increase in internal AChR complexes. Surface-expressed AChRs were equally stable in treated and untreated cells, however, differences were observed in the rate of increase in surface AChR. The rate of synthesis was increased 1.5-fold for α1 and 2.5-fold for α2. Subunit half-lives were increased 2-fold for α1 and 3-fold for α2. CPT-cAMP induced a proportional increase in the number of surface AChRs, a proportional increase in internal AChRs, and no change in the stability of surface AChRs. The rate of synthesis of only the α1 subunit was altered (2-fold increase) but the half-lives of all four subunits were increased 2-fold. Thus, forskolin appears to increase surface expression of AChRs by increasing the rate of subunit synthesis and increasing subunit stability while CPT-cAMP appears to only increase subunit stability. These results suggest that stimulation of surface AChR expression by PKA is regulated, at least in part, by posttranslational mechanisms.

FORSKOLIN DEPRESSES CAN CURRENT IN SNAIL NEURONS. L. Donald Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, NM 87113.

An important role for intracellular Ca2+ as a second messenger is in the activation of a class of calcium-activated non-specific cation (CAN) channels. These channels are found in a wide range of cell types where they either maintain depolarization or evoke secretion. (Partridge, L.D., Sandwulla, D., 77NS 11:69-72) Different stimulators of cAMP-dependent protein kinase (PKA) were used to investigate the regulation of expression of Torpedo nicoitonic acetylcholine receptors (AChRs) stably expressed in mouse fibroblast cell lines [Claudio et al. (1987) Science 238:1688]. As assayed by [3H]-bungarotoxin binding, an increase (1.5-3 fold) in surface AChR was detected after treatment with 3 different PKA stimulators: forskolin (10-100 mM), chlorophenylthio (CPT) cAMP (0.5-10 μM), and cholera toxin (100 μM). Forskolin treatment resulted in a 1.5-3 fold increase in surface AChR and a proportional increase in internal AChR complexes. Surface-expressed AChRs were equally stable in treated and untreated cells, however, differences were observed in the rate of increase in surface AChR. The rate of synthesis was increased 1.5-fold for α1 and 2.5-fold for α2. Subunit half-lives were increased 2-fold for α1 and 3-fold for α2. CPT-cAMP induced a proportional increase in the number of surface AChRs, a proportional increase in internal AChRs, and no change in the stability of surface AChRs. The rate of synthesis of only the α1 subunit was altered (2-fold increase) but the half-lives of all four subunits were increased 2-fold. Thus, forskolin appears to increase surface expression of AChRs by increasing the rate of subunit synthesis and increasing subunit stability while CPT-cAMP appears to only increase subunit stability. These results suggest that stimulation of surface AChR expression by PKA is regulated, at least in part, by posttranslational mechanisms.

FORSKOLIN DEPRESSES CAN CURRENT IN SNAIL NEURONS. L. Donald Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, NM 87113.

Forskolin was used to assess the role of adenylate cyclase in the modulation of Ca2+ currents in snail neurons. CAN currents in snail neurons have not been shown previously to be subject to modulation. Forskolin was used to assess the role of adenylate cyclase in the modulation of Ca2+ currents in snail neurons. Application of 10 μM forskolin (Sigma) rapidly and reversibly reduced CAN current (measured as total current area in ma x msec) by about 50%. Since serotonin appears to have the same effect on CAN currents, the mechanism involving G-proteins is implicated in the modulation of these currents. CAN currents in snail neurons have not been shown previously to be subject to modulation.


Recent data suggest that a phosphorylation process is involved in maintaining the function of the GABA receptor (Stelzer A.S., Kay A.R. and Wong R.K.S. 1988). We have now applied an intracellular patch clamp system to further characterize the intracellular modulation sites for the receptor. Purification was performed by extracting cell-culture Ca2+ dependent Ca2+ receptors from acutely dissociated hippocampal neurons. Adult guinea pigs were used for the experiments. As previously reported, GABA-induced outward currents were activated and maintained when ATP (2mM) or ATP (2mM)+Mg2+ (2mM) were present in the recording pipette (stable solution). Addition of alkaline phosphatase (100 μg/ml) to the stable solution induced run down of the current. The current was not clearly detected above the baseline noise. In addition we observed that stable responses were also obtained when ATP was substituted by ATP-S. GABA responses stabilized under this condition were more resistant to the phosphatase action.

Elevation of Ca2+ in the stable intracellular solution also caused reversible, rapid rundown of the GABA response. The run down in high Ca2+ was significantly reduced when GABA responses were first stabilized in ATP-S containing solution. In addition, a specific blocker of calmodulin also slowed the Ca2+ induced rundown. Our results are consistent with the suggestion that the elevation of intracellular Ca2+ destabilized the function of the GABA receptor by stimulating the dephosphorylation process.

INTRACELLULAR INJECTION OF INOSITOL HEXAKISPHOSPHATE INDUCES A BIPHASIC CURRENT IN IDENTIFIED NEURONS OF Aplysia. M. Sawada, W. Ichinose* and T. Maeno*. Dept. Physiol., Shimane Medical Univ., Izumo 693, JAPAN.

The ionic mechanism of the effect of intracellularly injected inositol hexakisphosphate (IP6) on the identified neuron of the Aplysia (Calciopila) was investigated with voltage-clamp, pressure-injection, and ion exchange techniques. Injection of IP6 into a neuron voltage-clamped at −55 mV produced a biphasic membrane current consisting of the first inward current (IIP1) followed by an outward current (IIP2) associated with increases in conductance. The peak of the inward current was dependent on both IP6 and IP6, and the peak of the outward current was dependent on IP6, IP6, and IP6. Neither IIP1 nor IIP2 was sensitive to TTX. Furthermore, neither IIP1 nor IIP2 was blocked by 4-AP and perfusion current dependent on IP6 was completely abolished in Ca2+-free plus 2 mM Ca2+ seawater but IIP2 was partially reduced in this solution. The biphasic currents characterized here would lead to potent modulation of neuropeptide neurons (89-812) of Aplysia by IP6.
A novel neuroactive peptide from Lycosid spider venom. J.B. Fischer, C.M. Miller. Brandeis University, Cambridge, MA 02139. When the peptide was added to a planar lipid bilayer membrane, voltage-dependent channel activity was observed. The effects were irreversible. The peptide completely blocked generation/propagation of action potential in rat phrenic nerve. H. Meiri and B. Gross, Dep. of Biology, Univ. Illinois College of Medicine, Chicago, IL 60612. The antiepileptic and anticonvulsant agents phenytoin, baclofen, memantine, and tizanidine have been examined in cultured spinal cord neurons of fetal mice. Effects of these substances on hyperexcitability were studied by tetanus toxin and picrotoxin in membrane current and were compared. Spontaneous activity of neurons was recorded by intracellular microelectrodes (700 100 MΩ). Application of tetanus toxin, strychnine and picrotoxin induced a hyperexcitability of the neurons by blocking inhibitory synaptic transmission in the neuronal network, which was increased by the presence of the inhibitors. Phenylpiracetam, J. Neurophysiol. 57:121-128, 1987). Phenylpiracetam, phenytoin, and tizanidine decreased the duration of bursts and the frequency of action potentials (AP) within the bursts, depending on the applied concentration. In contrast, baclofen decreased the frequency of bursts while leaving their duration and the frequency of AP within the bursts unaltered. Inward and outward currents were recorded by voltage clamp technique in the "Whole Cell" mode. Similar to phenytoin, memantine and tizanidine decreased the peak inward current over the voltage range from -120 mV to +100 mV. In contrast, baclofen decreased the peak outward current only in the voltage range from -20 mV to +100 mV. The currents were restored by switching to drug-free superfusion. Conclusion: the decrease of the current induced by baclofen reflects an activation of a potassium outward current, whereas the other agents directly block the sodium inward current. In addition, voltage dependent inactivation of the sodium inward current was shifted towards more negative potentials, thus leading to an earlier inactivation of inward current in the course of an AP and to an delay on its axonal propagation.
514.15
We have previously shown with ion selective microelectrodes that, in low pH-induced failure, uncoupling of crayfish septate axons, junctional resistance (Rj) and [Ca++]i curves match well with each other while Rj and [Ca++]i curves do not match. We suggested a Ca+-mediated gating mechanism (Perachia, C., Biophys. J., 55:151a, 1989). To investigate the mechanism of [Ca++]i increase we have studied the effects of different Ca-channel blockers and drugs that affect Ca-release from stores. Neither changes in Ca++ nor Ca-channel blockers had any effect. In contrast, caffeine affected both Rj and [Ca++]i maxima reached with acetate(AC)-induced low pH. Superfusion with Ac-salines containing 30 mM caffeine increased substantially the Rj maxima, while exposure to caffeine or cyanidol (1 µM) before, during and after Ac superfusion reduced significantly the Rj maxima with Ac type. Preliminary experiments with Ca-microelectrodes show that, with caffeine, [Ca++]i curves match well with Rj curves. These data indicate that cytoplasmic acidification closes channel gates via Ca++ released from internal stores. Vesicles (70 nm) coating both junctional surfaces could be Ca++ storing organelles providing the means for both Ca++ buffering and Ca++-release (Perachia, C. and Dulhunty, A. J. Cell Biol., 70:419, 1976). Sup. by NIH Gr. RO1113.

515.1
Based upon results from competitive inhibition studies and functional tests, 4-DAMP was originally classified as a selective muscarinic-M3 antagonist. Using membrane binding and autoradiographic techniques, we attempted to characterize the binding of 3H-4DAMP in the rat brain. In homogenates of various brain regions, 3H-4-DAMP appears to bind two classes of muscarinic sites, one with high affinity (Kd=0.14-0.53 nM) and low capacity (Bmax=19-31 fM) ANTAGONIST, IN RAT BRAIN. R. Quirion, D. M. Araujo, & P. A. Lepchok. Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

515.2
REGIONAL DIFFERENCES IN THE BINDING OF 4-DAMP TO RAT BRAIN COMPARISON WITH MINIMUM ENERGY CONFORMATIONS. D. A. Collins*, P. J. Smith* and W. S. Messer. Jr. (SPON: C.L. Hinman) Dept. of Medicinal and Biological Chemistry, College of Pharmacy and Dept. of Chemistry, Univ. of Toledo, 2801 W. Bancroft St., Toledo, OH 43614.
The binding of the muscarinic antagonist 4-DAMP, which is M3-selective in the periphery, was examined through quantitative autoradiographic techniques in brain. The density of the 4-DAMP binding sites in various areas of rat brain sections was compared with the known distribution of M3 and M4 muscarinic receptor subtypes as measured previously with pirenzepine and AF-DX 116. 4-DAMP displayed a high affinity for [3H]-QNB binding sites in rat brain sections. Analysis of 4-DAMP binding to various regions of rat brain revealed heterogeneous binding profiles, suggesting an interaction with multiple receptor subtypes.

515.3
RADIOLIGAND BINDING CHARACTERIZATION OF MUSCARINIC RECEPTOR SUBTYPES IN CEREBRAL BLOOD VESSELS. A.L. Collins*, D.A. Smith* and W.S. Messer. Jr. (SPON: C.L. Hinman) Dept. of Medicinal and Biological Chemistry, College of Pharmacy and Dept. of Chemistry, Univ. of Toledo, 2801 W. Bancroft St., Toledo, OH 43614.
Muscarinic cholinergic receptor sites in bovine cerebral arteries were analyzed by using radioligand binding assays with the specific muscarinic antagonist [3H]-quinuclidinyl benzilate ([3H]-QNB) as ligand. Specific binding of [3H]-QNB to membrane preparations from pial arteries was saturable, of high affinity (Kd=0.46±0.10 nM) and inhibited by the muscarinic antagonists atropine, 4-diphenylacetoxy-N-methylpiperidine metho-bromide (4-DAMP), dicyclomine, 11-(diethylnlamino) methyl-1-piperidinyl)acetyl-5,11-dihydroxy-6H-Camptothecin [CPT] and adenosine (AF-DX 116) and pirenzepine. The order of potency for the two classes of sites, with pirenzepine exhibiting greater potency at the lower affinity sites. Moreover, autoradiographic analysis of 3H-4-DAMP binding reveals dense labeling in the deep and superficial cortical layers, the hippocampal CA1 and dentate gyrus regions, and the external plexiform layer of the olfactory bulb, a pattern somewhat reminiscent of muscarinic-M3 binding in these areas. However, moderate to dense labeling was also observed in other brain structures, such as brainstem nuclei, which are not enriched with muscarinic-M1 sites. In summary, it appears that 3H-4-DAMP may be a useful ligand to study both the M1 and the M3 subtype of muscarinic receptor.

515.4
MUSCARINIC RELAXATION OF THE CAT MIDDLE CEREBRAL ARTERY IS MEDIATED BY M3 RECEPTORS. F. Daughn and F. Hamel, Laboratory of Cerebrovascular Research, Montreal Neurological Institute, Montreal, Quebec, H3A 2B4.
The subtype of muscarinic receptor involved in the endothelium-dependent relaxation to acetylcholine (ACH) was pharmacologically characterized in the cat middle cerebral artery. Cholinergic agonists such as carbachol, ACh and methacholine elicited maximal relaxations (Ecaum) which corresponded to 75-96% of the induced tone. Oxytocin and the M1 agonist McN-A-343 were significantly less potent vasoconstrictors (Ecaum of 31% and 24% of the tone, respectively).

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Recent pharmacological and molecular evidence indicates that up to four distinct muscarinic receptor subtypes may exist in the mammalian brain. Pharmacological classification of these receptors can be defined by their affinities for certain muscarinic antagonists. The various actions of muscarinic agonists in hippocampal neurons may be mediated by multiple receptor subtypes, although there is little quantitative data on this point. To test the hypothesis we used Schöll pilot study that separates agonists and antagonists. We studied the carbachol-induced input resistance in hippocampal neurons with intracellular recording in the rat hippocampal slice.

Following determination of a limited dose-response curve for carbachol in a cell, alternating doses of antagonist and agonist were given to determine antagonist- and agonist-induced rightward shifts in the dose-response relationship. At sufficient doses all antagonists blocked the increase in input resistance. The block was in turn overcome by higher doses of agonist. Schöll analysis revealed affinities of 7 nM for [3H]pirenzepine and 2 nM for AF-DX-116. The order of potency and quantitative values are in close agreement with the antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.

THE BINDING PHARMACOLOGY OF VARIOUS MUSCARINIC RECEPTOR ANTAGONISTS AND AGONISTS AT M3 RECEPTORS EXPRESSED IN M12 CELLS AND RAT BRAIN CORTEX. T.S. Smith, S.J. Edmond, F.J. Ehlert and F.M. Leslie, University of California, Los Angeles, CA 90024.

The binding pharmacology of representative muscarinic receptor antagonists and agonists at cloned M3 receptors was compared with binding results using rat cerebral cortex membranes as an "M3" receptor source. Binding inhibition curves for test agents were determined against [3H]-quinuclidinyl benzilate (QNB) labeled receptors. The rank order of antagonist binding potencies did not differ between receptor sources. Antagonist affinities were about 2 fold higher at cloned M3 receptors compared to cortical receptors. The lower rank order of agonist binding at cloning receptor source. Contrary to some earlier findings, we found that non-M1, non-M2 muscarinic sites.

The striae M3 displayed Kd values of 155 nM for AFXD, 0.2 nM for 4-DAMP, 14 nM for HSD, and 47 nM for MET. Similar values for AFDX and 4-DAMP were determined in the cortical M2R. However, differences between cortex and striatum were observed with MET and HSD. These data indicate that the M3R in both the striatum and cortex are non-cardiac.

COMPARISON OF THE BINDING PROFILE OF ALLOSTERIC ANTAGONISTS AT THE MUSCARINIC RECEPTORS OF RAT BRAIN. Uzoma N. Ude and E.S. El-Fakahany. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

The binding pharmacology of representative muscarinic receptor antagonists and agonists at cloned M1 receptors was compared with binding results using rat cerebral cortex membranes as an "M1" receptor source. Binding inhibition curves for test agents were determined against [3H]-quinuclidinyl benzilate (QNB) labeled receptors. The rank order of antagonist binding potencies did not differ between receptor sources. Antagonist affinities were about 2 fold higher at cloned M1 receptors compared to cortical receptors. The lower rank order of agonist binding at cloning receptor source. Contrary to some earlier findings, we found that non-M1, non-M2 muscarinic sites.

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A 639 base pair cDNA encoding 213 amino acids (Arg-134 to Lys-346) of the m1 muscarinic acetylcholine receptor (m1 MACHR) was isolated from a λgII1 rat brain library using a 40 base oligonucleotide probe. This cDNA was subcloned into pBR322, and after digestion of the construct with Sma I, an Eco R1 linker was introduced at Arg-220. Using Eco R1, a fragment encoding 137 amino acids of the third intracellular loop of the M1 MACHR was obtained and ligated into pl-RT123, a vector producing fusion proteins consisting of a truncated hMAChR (A) and the peptide corresponding to the introduced cDNA.

We plan to fuse a protein containing the 127 amino acid peptide of the third intracellular loop of the m1 MACHR. In this vector, the N-terminus of the MACHR is expressed in the presence of a heterologous protein (SPA) and the peptide corresponding to the introduced cDNA.

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Recent studies by Eber et al (FASEB J. 1: A1285, 1989) have indicated that [3H]-methylIocaine (NMS) binding to membranes of the rat corpus striatum is best explained as a model consisting of at least three populations of muscarinic binding sites. While the M1 and M2 muscarinic receptors can be labeled by pirenzepine (PZ) and AF-DX-116, respectively, these two sites only account for 37% of the total sites labeled by [3H]NMS. We have used radioligand binding pharmacology of muscarinic antagonists and agonists to M1 receptors expressed in M12 cells and rat brain cortex. The rank order of antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.

This binding can be completely displaced by 1 µM atropine, indicating that [3H]NMS binding pharmacology of muscarinic antagonists and agonists to M1 receptors expressed in M12 cells and rat brain cortex. The rank order of antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.


Recent pharmacological and molecular evidence indicates that up to four distinct muscarinic receptor subtypes may exist in the mammalian brain. Pharmacological classification of these receptors can be defined by their affinities for certain muscarinic antagonists. The various actions of muscarinic agonists in hippocampal neurons may be mediated by multiple receptor subtypes, although there is little quantitative data on this point. To test the hypothesis we used Schöll pilot study that separates agonists and antagonists. We studied the carbachol-induced input resistance in hippocampal neurons with intracellular recording in the rat hippocampal slice.

Following determination of a limited dose-response curve for carbachol in a cell, alternating doses of antagonist and agonist were given to determine antagonist- and agonist-induced rightward shifts in the dose-response relationship. At sufficient doses all antagonists blocked the increase in input resistance. The block was in turn overcome by higher doses of agonist. Schöll analysis revealed affinities of 7 nM for 4-DAMP, 280 nM for pirenzepine, and 2 nM for AF-DX-116. The order of potency and quantitative values are in close agreement with the antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.

The potasium channel blocker, 4-amino pyridine (4AP) bound with two high affinity sites in hippocampal neurons may be mediated by multiple muscarinic receptor subtypes, although there is little quantitative data on this point. To test the hypothesis we used Schöll pilot study that separates agonists and antagonists. We studied the carbachol-induced input resistance in hippocampal neurons with intracellular recording in the rat hippocampal slice.

Following determination of a limited dose-response curve for carbachol in a cell, alternating doses of antagonist and agonist were given to determine antagonist- and agonist-induced rightward shifts in the dose-response relationship. At sufficient doses all antagonists blocked the increase in input resistance. The block was in turn overcome by higher doses of agonist. Schöll analysis revealed affinities of 7 nM for 4-DAMP, 280 nM for pirenzepine, and 2 nM for AF-DX-116. The order of potency and quantitative values are in close agreement with the antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.
FUNCTIONAL EXPRESSION OF A MUSCARINIC CLONING, EXPRESSION, LOCALIZATION AND CONTROL OF THE ZINC-INDUCIBLE METALLOTHIONEIN PROMOTER AND EXPRESSED IN THE Y1 ADRENOCAINOMA CELL LINE. The cloned receptor is able to bind a variety of muscarinic specific agonists and antagonists and has a high affinity for quinuclidinyl benzilate and atropine and a low affinity for the muscarinic antagonist gallamine. Activation of the receptor by the agonist carbacol increases the production of total inositol phosphates and the level of cAMP accumulation in intact cells. In situ hybridization of the receptor gene to Drosophila salivary gland chromosomes localizes the gene to the distal end of the right arm of chromosome 2. Analysis of a genomic clone of the gene reveals that there is an intron located in the third cytoplasmic loop of the receptor.

SECRETED FACTORS REGULATE EXPRESSION OF AVIAN RETINA mAChR SUBTYPES IN VITRO. A.F. Subiria and V.L. Klein, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

Muscarinic acetylcholine receptors (mAChR) exist in the chicken central nervous system in two molecular weight subtypes (46kD and 72kD), and the relative proportions of these shift during synaptogenesis in vivo. In culture, the shift occurs in high density but not low density monolayer cultures, supporting the hypothesis that cell-cell interactions are involved. Further data support a role for secreted factors. Transfer of high-density cultures, placed on coverslips in larger plates containing a greater volume of media decreased the expression of the mature 72kD form; this expression was restored by growing the cultures in cell density conditioned media, indicating that a secreted factor is responsible for its down-regulation. Using low density cultures, the expression of both subtypes is stable, supporting the hypothesis that cell-cell interactions are required to cause these changes in receptor expression. These data suggest that developmentally-regulated secreted factors in the culture media may be responsible for the shift in mAChR subtypes expression seen during in vivo development.

DESENSITIZATION AND SEQUESTRATION IN TWO MUSCARINIC RECEPTOR SUBTYPES. J. Baumeold and B. Cooperman. Lab. of Neurobiology, NINDS, Bethesda, MD 20892.

Muscarinic acetylcholine receptors (mAChR) are involved in a wide variety of cellular functions. In the present study, we have examined the capacity of the MAChR subtypes to desensitize and sequester in a cell line (PC12) derived from the pheochromocytoma PC12 rat adrenal gland. The mAChR subtypes were distinguished by their kinetics of desensitization and sequestration. The mAChR subtypes were able to desensitize and sequester in a biphasic manner described previously for cardiac receptors (Eliis and Zeidenberg, Mol. Pharmacol. 39:173, 1989). There was a significant difference in the rate of desensitization between low affinity (1.9 x 10^5 M^-1 s^-1) and high affinity (1.1 x 10^5 M^-1 s^-1) PZ binding sites. These results suggest that the MAChR changes in response to agonist binding in a manner that may be related to the development of tolerance to the biological activities of acetylcholine.

Recent studies suggest that cell surface receptor stimulation by neurotransmitters can elicit a rapid genomic response that help define the neurotransmitters mediating this response. We have examined muscarinic receptor activation of Hotel, a putative transcription factor gene, in NIE-115 neuroblastoma cells. This cell line contains two pharmacologically-defined muscarinic receptors: "M2"-like receptors linked to reduction of cyclic AMP levels, and "M1"-like receptors linked to stimulation of cyclic AMP synthesis and PI turnover. The "M" receptor-specific agonists oxotremorine and arecoline rapidly increase Hotel mRNA levels in NIE-115 cells, with mRNA reaching maximal levels within one hour. The induction is blocked by 2 µM atropine, a muscarinic antagonist. Induction of Hotel mRNA by oxotremorine (50 µM) is also blocked by pre-treatment of the cells with 5 ng/ml perctusis toxin for 18 hours. These data suggest that muscarinic receptor-mediated activation of Hotel involves high affinity interactions of the first-messenger levels in a cellular system and is mediated by activation of the M2-like receptors via a perctusis toxin sensitive G protein.

EFFECT OF GTP AND GppNHp ON AGONIST-INDUCED COUPLING OF MUSCARINIC RECEPTORS (mAChR) TO G PROTEINS, D.F. Mataesic* and G.R. Luthin (Spon: G.C. Saladram). Dept. of Physiology & Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

A series of analogues of the muscarinic agonist BMS were synthesized using the functionalized congener approach to drug design (Jacobson et al., Mol. Pharmacol. 22 126, 1986). These compounds were evaluated for agonist activity at the second-messenger level in a series of cell lines that express a single muscarinic receptor subtype. Compound BMS was found to stimulate only the adenylate cyclase-coupled muscarinic receptor, M1 and M3. Therefore, BMS is a subtype-selective muscarinic agonist. Since BMS has been described as a post-synaptic agonist and a pre-synaptic antagonist (Nordstrom et al., Mol. Pharmacol. 26 1-5, 1985), these studies indicate that post-synaptic muscarinic receptors are adenylate cyclase coupled, whereas pre-synaptic muscarinic receptors are coupled to PI turnover. Like BMS, several of the novel compounds were also subtype-selective partial agonists in that they stimulated only the M2 and M4 subtypes and were inactive as agonists at M1 or M3 receptor subtypes. These compounds are being evaluated further for their potential in treating the cognitive deficits of Alzheimers disease.

MUSCARINIC RECEPTOR-G PROTEIN INTERACTIONS IN HEART. G.R. Luthin and D.F. Mataesic*. Dept. of Physiology and Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

Membranes from rat heart were incubated with muscarinic cholinergic receptor (mAChR) agonists or antagonists, then solubilized using digitonin/cholate. mAChRs purified by WGA affinity chromatography were precipitated using a cardiac-selective anti-mAChR antibody (Luebbe et al., Biochem 26:6892, 1987). When mAChRs were thus isolated from cardiac (10 nM)-labelled membranes, the 39 kDa alpha subunit of Go was found to copurify with the mAChRs. The Go alpha subunit did not copurify with atropine-labelled mAChRs. In the presence of 20 mM NaF, the concentration of cardiac Go, both 39 kDa and 40 kDa Go alpha subunits copurified with mAChRs. Saturation concentrating of agonists with full (cardiac), partial (pilocarpine) or no (McNA343) efficacy in the heart adenylyl cyclase assay all promoted copurification of G alpha subunits with mAChRs, though to varying levels. These results demonstrate a novel method to investigate mAChR coupling mechanisms. (Supported by NS23006).
ABSENCE OF AGE-RELATED ALTERATION IN BRAIN MUSCARINIC RECEPTOR-MEDIATED PHOSPHOSTIGMIDE HYDROLYSIS AND IN ITS ALTERATION DUE TO NERVE ENLSTERS, ETBROXIN, AND RECEPTOR DESENSITIZATION. U. Suritcherm, E.A.M. Abdellah, and E.E. El-Fakahany. Dept. of Pharmacol. & Toxicol., Univ. of Maryland School of Medicine, MD 21201

Phosphostigmine (PI) hydrolysis products have been postulated to play important roles in regulating neuronal function. The presence of a Ca2+ process of mem- brane by age. Therefore, the effects of aging on muscarinic receptor-mediated PI hy- drolysis in various brain regions were investigated in male Fisher-344 rats. The muscarinic agonist oxotremo- rine-M induced a dose-dependent increase in this response to the same magnitude in the cerebral cortex, striatum, hippocampus, thalamus, hypothalamus and cerebellum in young and old animals. The E50 measured in the first three brain regions were independent of age. The ability of elevated K+ to potentiate the response in cortex was equal in both age groups. In addition, a phorbol ester and tetrodotoxin suppressed the agonist-induced PI hydrolysis in cerebral cortex, striatum and hippocampus to the same extent in young and old rats. Moreover, there was no influence of aging on down-regulation of cell surface receptors and desensitization of receptor function in cerebral cortex after preincubation with oxotremorine-M. Therefore, PI hydrolysis in response to activation of brain muscarinics does not appear to be sensitive to aging-related alterations.

DIFFERENTIAL EFFECTS OF CALCIUM ANTAGONISTS ON RAT BRAIN MUSCARINIC RECEPTOR SUBTYPES. M. Watson and X. Mina*.

To study functional and topographical relationship between muscarinic receptor and calcium channel, we investigated interactions of various calcium antagonists such as nicardipine, verapamil and diltiazem with muscarinic receptors using a receptor binding assay technique. Experiments were done using [3H]-QNB as a muscarinic ligand. Tissue homogenates were obtained from male Wistar-strain rats weighing 180-220 g.

Nicardipine, verapamil and diltiazem inhibited [3H]-QNB binding completely. Displacement curves of [3H]-QNB binding by nicardipine (2.3 nM) and diltiazem (1.1 nM) indicated that the affinity of nicardipine remained at higher concentrations of the ligand. The Schild plot of nicardipine yielded curvilinear functions. This deviation from linearity of the Schild plot indicated possible allosteric interaction between muscarinic receptor and nicardiazem binding sites. Those of verapamil and diltiazem showed linearity with slope of 1.0 and 1.5, respectively.

DIFFERENTIAL RESPONSIVENESS TO BM-5 (N-METHYL-N-[1-METHYL-4-PYRROLIDINO-2-BUTYL]IACETAMIDE AND CARBAMYLCHOLINE IN MUSCARINIC RECEPTOR INDUCED HYPOThERMIA AND BINDING TO RAT BRAIN MUSCARINIC RECEPTORS. T. Hohsaka, S. Tsukita and K. Miyoshi.

We have used voltage-sensitive indicator dyes in conjunction with flow cytometry to characterize the responses of A9 fibroblasts, stably transfected with m1 muscarinic receptor cDNA, to cholinergic agents. Cells were removed from flasks with trypsin and allowed to recover for 2 h at room temperature in physiological saline. Cells were stained sequentially with the anionic dyes DBIC(A)-H and the cholinergic ligand; the fluorescence (FL) intensity of 10-20,000 events was analyzed on a Becton-Dickinson FACCS 440. The muscarinic agonist acetylcholine (ACH) and carbachol, at concentrations ranging from 10-5M to 10-3M, caused a decrease in FL of more than 50% in both o xo FL indicating relative hyperpolarization of the cells. These responses were blocked by the muscarinic receptor antagonist atropine (1µM) but not the nicotinic receptor antagonist tubocurarine (5µM). Tubocurarine and atropine alone had no effect. Ion substitution experiments replacing sodium with N-methyl-D-glucamine resulted in a 30-35% decrease in oxo FL indicating relative hyperpolarization of cells. These results are in agreement with those obtained using patch-type electrophysiological recording techniques (Jones et al., PNAS, 85:6056, 1988). Thus, flow cytometric analysis of newly transfected cells can be used to determine the success of transfection for neurotransmitter receptors or other membrane proteins functionally coupled to membrane potential. The sorting capability of the instrument should make it possible to enrich for transfected cells without the necessary time and effort involved to establish neomycin resistance.
516.1

Effect of chronic haloperidol treatment on rat striatal calcineurin (CN) activity. E. Chung, M.T. Dvoroznak*, M.H. Van Woert and H.C. Li*.

Department of Neurology, Pharmacology and Biochemistry, Mount Sinai School of Medicine, New York, NY 10029.

CN activity is a Ca2+-calmodulin-dependent phosphatase present in highest concentrations in the striatum. It has been suggested that CN regulates neurotransmission by dephosphorylating the [Ca2+/calmodulin] signal. We have shown that CN activity is localized to intrinsic striatal neurons. Some striatal neurons contain DA receptors and receive nigral DA input. Since DA receptors are linked to adenyl cyclase we have investigated whether rat striatal CN activity (measured using 32P-ser-casein) is altered by DA receptor supersensitivity produced by haloperidol injections for 20 days.

Control activity 3H-spiroperidol

<table>
<thead>
<tr>
<th>Control</th>
<th>1.71±0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol 0.5 mg/kg</td>
<td>148.6±7.4</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>124.0±6.8</td>
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</tbody>
</table>

* p < 0.001 compared to control.

516.2

Effect of quinolinic acid lesions (QA) on rat striatal calcineurin (CN) activity. M.H. Van Woert, E. Chung, M.T. Dvoroznak* and H.C. Li*.

Department of Neurology, Pharmacology and Biochemistry, Mount Sinai Sch of Med, New York, NY 10029.

Intrastriatal infusion of QA, an endogenous excitotoxin, is thought to mimic the neurochemical and histopathological features of Huntington's Disease. We have investigated the effects of varying doses of QA on rat brain calcineurin activity (measured using 32P-ser-casein). In order to estimate the extent of intrinsic neuronal cell body loss, GAD and CAT activities in the same striatal tissue were also measured.

Control activity GAD CAT

<table>
<thead>
<tr>
<th>Control</th>
<th>1.63±0.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>QA 30 nmoles</td>
<td>1.14±0.09*</td>
</tr>
<tr>
<td>QA 90 nmoles</td>
<td>0.76±0.05*</td>
</tr>
<tr>
<td>QA 270 nmoles</td>
<td>0.79±0.07*</td>
</tr>
</tbody>
</table>

* p < 0.001 compared to control.

516.3


We have measured the in vivo activity of the electric organ [Na+, K+]ATPase in the dorsal membrane of the electrocute of Mabrincean brasilienis noninvasively using 31P and 23Na nuclear magnetic resonance spectroscopy. Activation of the sodium pump induced by electrical stimulation of the organ can be accurately quantitated by monitoring the fall of phosphocreatine (PCr) and a high energy phosphagen in the electrocute. This depletion is blocked by preincubation in the cardiac glycocide ouabain. The [Na+, K+]ATPase rate increases by a factor of at least 2000 with normal stimulation so as to maintain intracellular sodium ([Na+]i) unchanged until virtually full depletion of PCr. Recovery of [Na+]i is accomplished in under 2 sec. However, incubation of excised slices of electric organ under conditions that will increase [Na+]i, including hyperosmolar buffer, gramicidin, and mollenin failed to increase PCr turnover. In contrast, tetraphosphophonium bromide, which collapses the transmembrane potential, does activate the [Na+, K+]ATPase, even after preincubation in the nicotinic antagonist d-quinacrine chloride. It thus appears that activation of the sodium pumps in this tissue may depend upon, e.g., association of the ventral membrane rather than sodium concentration alone. The results suggest that Mabrince may be an excellent model for study of Na+ pump activation in an excitable tissue.

516.4


Rat forebrain Ca2+-Kinase II in isolated postsynaptic densities (PSD) was subjected to limited proteolysis by μ-calpain, a Ca2+-dependent protease. Incubation of autophosphorylated or Ca2+/CaM-bound kinase with this protease resulted in solubilization of a Ca2+-/CaM-dependent kinase activity which was 3-5 fold greater than the initial Ca2+/CaM-independent activity in the PSD. Western blot analysis using polyclonal antibodies to soluble kinase holoenzyme indicated that μ-calpain generated several immunoreactive fragments between 21-30kDa. μ-Calpain, however, only degraded a small fraction of the intact kinase subunits. [32P]Ca2+-overlays indicated a major Ca2+-binding fragment of 29kDa in μ-calpain digests of CaMK-II. This peptide was shown to contain the regulatory autophosphorylation site (Thr-286) of the kinase. Immunoblotting with antibody raised to a synthetic peptide from the catalytic domain of the kinase indicated that there was a single active fragment of approximately 30kDa in the μ-calpain digests. Analysis of crude digest using Superose 6 gel filtration also indicated that Ca2+-independent kinase activity resided in a 30kDa fragment. Thus, μ-calpain appears to cleave CaMK-II into a 30kDa catalytic domain fragment and a 20kDa regulatory domain fragment. These data support a putative mechanism for persistent regulation of synaptic events by such proteolytic activation of CaMK-II.
516.5 A Ca2+-DEPENDENT, LONG-LASTING HYPERPOLARIZATION INDUCED BY INOSITOL TRISPHOSPHATE (IP3) IN CAT SPINAL MOTONEURONS. L. Zhang and K. Kraljevic. Dept. Anaesthesia Res. and Physiology. McGill University, Montreal, Quebec, Canada H3G 1Y6.

In experiments on pentobarbital-anesthetized cat or decerebrate在京神经元, trypsin digestion of IP3 (3-10 nM for 0.5-1 min) induced a reproducible, long-lasting hyperpolarization (2-16 mV), with a peak amplitude occurring at 0 mV. Hyperpolarization started 0.5-1 min after the injection and was maximal within 3-5 min and persisted for another 10-60 min. Associated with the hyperpolarization, there were increases in the spike size and rate of rise, and in EPSP amplitude, but the post-spike after-depolarization was unchanged or increased in amplitude. Repetitive discharges, evoked by intracellular depolarizing currents, were reduced during the hyperpolarization. Simultaneous injections of IP3 and BAPTA did not induce any hyperpolarization, whereas the Ca2+-dependent AMPH was depressed by 60%. The effects of IP3 were closely mimicked by the long-lasting hyperpolarizing action of 5-HT (probably mediated by 5-HT3 receptors) on these motoneurons, in their slow time course and Ca2+-dependence, suggesting that IP3 may be an intracellular mediator of 5-HT action. Supported by MRC of Canada.


Synaptic potentials and transfer impedance functions of the Müller giant axon terminal-synapsized spinal neuron were measured by placing one intracellular electrode near the pre-synaptic terminal and a second electrode in the soma of the postsynaptic spine. Measurements of the chemical synapse in zero calcium the axon remains electrotonically connected through gap junctions providing a direct access to the dendritic tree from the electrode in the axon. It was shown that the electrotonic synaptic potential was enhanced by depolarization of the soma. Similar depolarizations caused an increase in the membrane impedance using a white noise single electrode transfer function analysis. This effect was abolished by 1 µM TTX suggesting that a subthreshold voltage dependent negative sodium conductance is responsible for the enhancement of synaptic potentials with depolarization. A synaptic transfer function was used to estimate the synaptic location on an equivalent dendritic cable and calculate the synaptic potentials for different membrane resting states. Supported in part by NIH-ZP01-NS-11255.

516.7 AMINO ACID RESPONSES OF LAMPREY SPINAL NEURONS. J.T. Buchanan*, R.N. Christensen, and L.T. Moore (SPON: H.A. Leckman). Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Lamprey spinal neurons, either isolated or within the spinal cord, were tested with excitatory and inhibitory amino acids. The response of enzymatically isolated neurons to the excitatory amino acids quisqualate (QA) and N-methyl-d-aspartate (NMDA) were measured with whole-cell patch clamp electrodes. Both QA and NMDA induced an inward membrane current with a reversal potential near 0 mV indicating that they open cation selective channels. QA responses had an EC50 and Hill coefficient of 4.3 µM and 2.2, respectively, the latter indicating cooperativity in QA binding. Currents were reduced by extracellular Mg2+, and the current-voltage curve in normal Mg2+ displayed a negative-slope conductance near the resting potential. NMDA responses were potentiated by glycine with an EC50 and Hill coefficient of 1.4 µM and 1.7, respectively. Measurements of impedance functions with a white noise technique revealed that the excitatory amino acids glutamate and QA caused a slight decrease in the membrane impedance of both isolated neurons and intact motoneurons. In contrast, NMDA induced a pronounced increase in the impedance consistent with the negative-slope conductance observed in the NMDA current-voltage curve. The inhibitory transmitter glycine and GABA decreased the impedance over a wide frequency range. Supported by NS-1125 from NHHS.

516.9 POSSIBLE DUAL EFFECT OF SYNAPSES THAT ARE PU-TATIVELY PURELY EXCITATORY OR PURELY INHIBITORY: BASES IN STABILITY THEORY AND IMPLICATIONS FOR NEURAL NETWORK BEHAVIOR. R. Davenport*, E. Jakobson, and H. Gehr. (SPON: F. Delcours). Dept. of Physiol. and Biophys., Progs. in Neur. and Behav. Biol. and Bioeng., Univ. of Ill., Urbana, IL 61801.

Depolarization of an excitatory membrane has a dual effect: excitatory in that it causes rapid opening of calcium and/or sodium channels but inhibitory in that it also causes those channels to inactivate. We considered whether apparently paradoxical or dual behavior might be exhibited by excitatory and inhibitory synaptic inputs. We used the classic Hodgkin-Huxley model for voltage-gated channels and simulated hypotheses of appropriate excitatory, inhibitory, and ligand-gated post-synaptic channels. We summarize a model cell's behavior by calculating elicited firing frequency as a function of reversal potential and conductance of summed synaptic inputs, using stability theory and direct simulations. Dual behavior is elicited in the model when appropriate densities of ligand-gated channels. Thus a particular synaptic input to a neuron may be either excitatory or inhibitory depending on simultaneous activity of other synaptic inputs to the cell. This output-map technique may give rise to biologically realistic and rich behaviors as an element of composed neural networks, and still be computationally tractable.


We have characterized evoked and spontaneous synaptic currents in neocortical neurons with whole-cell voltage-clamp recordings. Visual and somatosensory evoked responses were at 30-40 day old rats were cut with a vibratome (400 µm), affixed to the bottom of petri dishes with plasma-thrombin clots, and superfused with artificial cerebrospinal fluid. Gigahm seals were formed by plunging recording pipettes (5 Megohms) into the slices that were held under a constant pressure, and advancing the electrodes slowly to form partial seals (100-200 Megohms). Seals were then applied through the electrodes to form gigahm (2-7 Gigohms) and additional suction was applied to rupture the underlying membranes and obtain whole-cell recordings. The input impedances for neurons recorded with this technique were 5-15 Gigaohms. Neurons were labeled intracellularly with either Lucifer Yellow CH or biocytin.

We recorded from both pyramidal and nonpyramidal neurons in layers II-VI. Spontaneous synaptic events occurred at high frequencies, were not reduced by 0.5 µM TTX, and could be resolved as single or multiple events. Most of the spontaneous currents reversed in direction at the chloride equilibrium potential and were blocked by bath application of 20-50 µM bicuculline methiodide (BMI). The spontaneous events that were still present in BMI reversed at or near the equilibrium potential for monovalent cations (44.5 mV). Simulation of the white matter, in 5 µM BMI, evoked a large conductance current that often had two components, and both reversed near 4 mV. Addition of 20 µM d-APV greatly reduced the duration and amplitude of the first component and blocked the second.

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516.11
WHOLE-CELL PATCH-CLAMP RECORDINGS IN ADULT RAT HIPPOCAMPAL SLICES. L. Mody and T. S. Ong, Dept. of Neurology and Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305.

The advantages of the patch-clamp technique have been exploited in thin (<130 µm) mammalian brain and spinal cord slices where neurons could be identified with the aid of Nomarski water immersion optics (Kosso et al., Soc. Neurosci. Abstr., 14, 1986). We have employed a simplified version of the above technique to achieve patch-clamp recordings in standard (100 µm thick) hippocampal slices prepared at 35±0.5°C in a conventional recording chamber.

Patch electrodes (2-2.5 µm diameter; 4 MΩ), usually filled with (in mM) K-mesylate-sublactate 120, MgCl2 2, HEPES 10 (pH 7.2), were lowered into any of the cell body layers of the slice under visual guidance through a dissecting microscope. Seals formed (>1 GΩ) within the upper 50 µm of the slice. Following breakthrough, an Axoclamp 2A was used for current- or voltage-clamp recordings. In over 30 CA1 pyramidal (PCs) and dentate gyrus granule cells (GCs) the resting membrane potentials (RMPs) ranged between -67-72 mV (PCs) and 68-80 mV (GCs); input resistances (Ri) were 70-130 MΩ (PCs) and 100-220 MΩ (GCs). All neurons had overshooting action potentials with a threshold of 40-50 mV more positive to rest. The RMPs are in good agreement with previous studies using sharp electrode recordings. However, the Rs are consistently 4-5× larger than those reported with conventional intracellular microelectrodes. This discrepancy can be attributed to the less invasive nature of the patch-clamp recordings which produce minimal damage to cell membranes, thus avoiding leak conductances.

In summary, the patch-clamp recordings can easily be adapted to conventional preparation and recording techniques in hippocampal slices. Based on its advantages, the patch technique is anticipated to replace sharp electrode recordings in brain slice preparations.

Supported by NIH grants NS12151 and RR03533-27 to I.M.

516.12
MEASUREMENT OF INTRACELLULAR CALCIUM BY FLUORESCENCE INTRAVITAL FLUORESCENCE MICROSCOPY IN PYRAMIDAL CELLS OF HIPPOCAMPAL SLICES. R. Delay, D. Hochman, and B.A. MacVicar (Sponsor D. Burnard), Department of Medical Physiology, University of Calgary, Calgary, Alberta.

Intracellular calcium is an important signal controlling neuronal excitability and probably has a role in inducing neuronal death during some neurodegenerative diseases. We have developed a method by which we can measure intracellular calcium in hippocampal pyramidal cells in vitro slices while simultaneously recording activity. This has also allowed us to determine the effects of intracellular calcium during spike activity and also during seizure activity in slices. Slices (300 µm) were isolated from the hippocampal level 180 µm of rats transferred to a recording chamber on an inverted microscope for staining and recording. Fura-2 (10 µM in 0.2M Kacetate, 10 mM HEPES, pH 7.2) was intracellularly injected into pyramidal neurons. Fluorescence images of cells were obtained using a SIT camera and digitized with 17 IS imaging analysis system. Fluorescence intensity ratios of cells at 360/380 or 430/430 wavelengths (N) were calibrated by comparison with either buffered Ca2+ solutions or after application of A-23187 to obtain Kmax. Bursting activity induced by either current injection or application of convulsants was correlated with increased dendritic Ca2+.

516.13

Clinically, hypomagnesemia produces, by an unknown mechanism, manifestations of central nervous system irritability. Experimentally, hypomagnesemia was simulated by sequential changes in external [Mg+] in in vitro slice preparations of somatosensory cortex. Mg-free perfusion induced a small hyperpolarization (3-5 mV) with a 10% decrease in input resistance, slow depolarizing waves insensitive to tetrodotoxin (TTX) and a TTX-sensitive enhancement of the NMDA were potentiated. Increasing external [Mg+] in slices that had been depolarized to -60 mV in Ca2+-free medium produced a 10-20 mV depolarization. Glutamate responses were not affected whereas responses evoked by acetylcholine and GABA and did not produce significant alterations of the glutamate responses. The results suggest that the effects of hypomagnesemia on transmitter actions may involve pre- and post-synaptic mechanisms.

516.14
FIRING PROPERTIES OF HUMAN NEOCORTICAL NEURONS. D. Frederick*, C. J. Wilson, A. R. Wyler* and R.C. Foehring (Spon: D.M. Desiderio). Depts. of Anatomy and Neurobiology, and Neurosurgery, Univ. of Tenn., Memphis, TN 38163.

Human neocortical neurons were studied in a slice preparation from the middle temporal gyrus. Intracellular recording revealed two classes of response to sustained current injection. Regular-spiking neurons (75% of cells) fired repetitively throughout a 1 s current injection, regardless of prior holding potential. Bursting neurons (25% of sample) responded to similar current injection by a burst of 2-3 spikes rising upon a slow depolarizing potential, then were quiescent. Bursting was a voltage-dependent behavior: neurons showed a burst response from holding potentials of >150 mV (negative to -70 mV) but fired repetitively when held at depolarized potentials. Bursting was also dependent upon stimulus intensity; at hyperpolarized potentials a large stimulus resulted in repetitive firing. Bursting neurons exhibited a larger afterdepolarization following an action potential than regular-spiking neurons (only significant difference). Several cells were filled with biocytin and later stained to determine neuronal morphology. Firing types were observed in pyramidal cells. Variability of firing pattern suggests that bursting and regular-spiking may be extremes of a continuum of firing behavior.

516.15

Excitatory post-synaptic potentials (EPSP's) produce an increase in the firing rate of active neurons by advancing the occurrence of threshold crossings in the interspike interval (ISI). We investigated the underlying mechanisms by probing the ISI with depolarizing pulse potentials (PP's), which have been shown to mimic the effects of EPSP's on neuronal firing.

Intracellular recordings were obtained from layer V pyramidal cells in slices of cat somatosensory cortex. To mimic EPSP's, brief current pulses were injected through the recording electrode, producing PP's with a near-linear rise of membrane potential followed by an exponential decay (in resting neurons). During repetitive discharge, induced by injection of d.c. current, the PP's were injected at random intervals. Offline computer analyses calculated the average ISI shortening produced by PP's at different delays and their contributions to the cross-correlograms.

PP's were found to shorten the ISI in two ways. During the last part of the ISI, the PP's crossed threshold (which is an increasing function of time) and produced counts in the correlogram peak. At earlier times in the ISI, the PP's produced delayed threshold crossings that also shortened the ISI, apparently due to a persistent sodium current; these PP's contributed to the cross-correlogram counts beyond the peak. Preliminary observations suggest that, in many cells, the second effect can contribute more to the overall change in firing rate than the direct threshold crossings.

516.16
INWARD RECTIFICATION VARIES WITH CORTICAL LAMINA IN GUINEA PIG NEOCORTICAL NEURONS IN VITRO. A. Williamson and D. A. McCormick* (Spon: M. Strichartz). Dept of Neurobiology, and Neurosurgery, Univ. of Calif., Irvine, CA 92717.

Two forms of inward rectification have been described in neurons: the first is a K+-dependent current which activates hyperpolarized to -65 mV and is sensitive to both extracellular Cs+ and Ba++. The second, known as inward rectification, is a mixed Na+-K+ current that activates at membrane potentials negative to -55 mV and can be readily blocked by Cs+ but not by Ba++. In this study we investigated the rectification properties of pyramidal cells in layers II-III and in layers V neurons isolated from the forelimb and visual cortical areas in guinea pig. Intracellular recordings were obtained using 2-3 mV spikes recorded using an inverted microscope for staining and recording. Fura-2 (10µM in 0.2M Kacetate, 10 mM HEPES, pH 7.2) was intracellularly injected into pyramidal neurons. Fluorescence images of cells were obtained using a SIT camera and digitized with 17 IS imaging analysis system. Fluorescence intensity ratios of cells at 360/380 or 430/430 wavelengths (N) were calibrated by comparison with either buffered Ca2+ solutions or after application of A-23187 to obtain Kmax. Bursting activity induced by either current injection or application of convulsants was correlated with increased dendritic Ca2+.

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516.17

DIAMID RECTIFICATION OF PROJECTION CELLS IN THE RAT AND CAT LATERAL GENICULATE NUCLEUS. B. Lightwood*, J.W. Hind*, and V. Crunelli. Dept. Pharmacology, St. George's Hospital Medical School, London SW1 0RE, UK. Thalamic projection cells in vivo and in vitro often display rapid membrane potential oscillations which are partly due to the activation of time- and voltage-dependent conductances. We have now studied the properties of a hyperpolarization activated current using the single-electrode voltage clamp technique in the thalamic lamina IV of the dorsal lateral geniculate nucleus (150-170mM Na+, 0.5-5mM Ca2+, 6.25 mM K+, 10 mM HEPES and 2.5 mM Mg2+). Hyperpolarization stage II holding potentials of -45 to -55mV evoked a slow non-inactivating inward current that was followed by a slow tail current on repolarization to the holding potential. The current was activated over the range -55 to -120mV and tail current analysis showed a reversal potential of -1.1 ± 1.9mV. This value was shifted to more negative potentials by reducing either [Na+]o or [K+]o and thus to more positive potentials by increasing [K+]o. The current was reversibly abolished by 3mM Ca2+ but relatively resistant to 6mM Ba2+. We conclude that thalamic projection cells possess a hyperpolarization-activated inward current carried by Na+ and K+ and these currents may play a role in the circuitry of thalamic projection cells.

516.19

NMDA AND NON-NMDA RECEPTORS MEDIATE BOTH HIGH AND LOW FREQUENCY SYNAPTIC POTENTIALS IN THE RAT LATERAL GENICULATE NUCLEUS. I. Soltesz*, M. Habv*, D. Jassik-Guran*, E. Lequeur*, and D. Pleyer*. Departments of Pharmacology and Anatomy, Faculty of Medicine, University of Geneva, Geneva, Switzerland. We have tested the relative contribution of NMDA and non-NMDA receptors to synaptic potentials evoked in pyramidal neurons of the lateral geniculate nucleus by low and high frequency stimulation of the optic tract. In the presence of 6-AP, 6-cyano-7-nitroquinolinoxaline-2,3-dione (CNQX) (5mM) and the NMDA receptor antagonist APV (10mM), reversible reductions (85%±8%) of the EPSPs evoked at low frequency stimulation (0.03-0.05Hz) were generated by discrete somatic or dendritic current sources and the field responses. In the same cells, however, APV applied before CNQX had no effect on the EPSP. High frequency stimulation (50-100Hz, 200-500 msec) evoked at each shock superimposed on a slow depolarization. APV reversibly reduced (45-80%) the slow depolarization with no effect on the fast EPSPs. These results indicate the involvement of both NMDA and non-NMDA receptors in the retinal input to the LGN and are consistent with results recently obtained in cat and 12 cells in vivo (Murphy, Salt & Sillito, Physiol. Soc. Meet., London, April 1989).

516.20

SIMULATION OF EVOKED FIELD RESPONSES. L. S. Leung*, Dept. Clin. Neurol. Sci. Physiology, Univ. Western Ontario, London, Canada N6A 5C1. Field potentials in a cortical structure (e.g. hippocampus) were simulated by a three-step procedure: (1) Intracellular responses using the Fahl's compartment model. (2) Current-source densities and then (3) field responses. This approach allows direct comparison with experimental data. The results indicate that the field potential is generated by an evoked current source. Simulations of the synaptic inputs represented by texp(-at) functions. Proximal dendritic excitation gave mirror-image like field potentials. The synaptic inputs were generated by excitatory inputs. The time course of the inhibitory input was assumed to be 10 times slower than the excitatory one. However, large open (dipole) fields were generated by somatic or dendritic IPSPs. Supported by NSERC and MRC.

CATECHOLAMINES

517.1

THE EFFECTS OF α-METHYL-D-TYROSINE ON THE CONCENTRATION OF β-PHENYLETHYLAMINE IN THE RAT STRIATUM. L.A. Paterson, A.V. Joppo* and M.Y. Zhu*. Neuropsychiatric Res. Unit, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0. β-Pheynethylamine (PE) is an endogenous brain amine which is present in the striatum at a concentration of 3.3 ng/g. PE is synthesized by the action of dopamine hydroxylase on dopamine or tyrosine (1.25 mg/kg, i.p., 2 hours) did not affect the concentration of PE in striatum but prevented the stimulation-induced increase in the concentration of PE. These experiments demonstrate that the rate of accumulation of PE is decreased by the activation of TH and therefore, PE is present in TH-containing neurons. We conclude that PE exists with DA in dopaminergic neurons in the nigrostriatal pathway. Supported by Saskatchewan Health and the MRC of Canada.

517.2

THE EFFECTS OF A BENDIZIAPEINE PARTIAL AGONIST, RO 16-6028, ON BASAL AND STRESS-INDUCED IN VIVO TYROSINE HYDROXYLATION IN THE MESOTELENCEPHALIC DOPAMINE SYSTEM. K. Birznieks*, A. Y. Deutsch* and N. Goldstein (POOH; J. E. Place). Department of Psychiatry, New York University School of Medicine, New York, NY 10016 and Dept. of Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06520. Anxiolytic benzodiazepine (BDZ) agonists decrease dopamine (DA) turnover in mesotelencephalic DA terminal fields, and prevent stress-induced increases of DA turnover in the prefrontal cortex (PFC). Ro 16-6028, a novel BDZ partial agonist, possesses the anticonvulsant and anxiolytic properties of typical BDZ agonists but exhibits greatly reduced muscle relaxant and sedative properties relative to full BDZ agonists (J. R. Martin et al., Pharmacologia. 271: 136, 1989). Ro 16-6028 was examined the effects of Ro 16-6028 on in vivo tyrosine hydroxylation in the mesotelencephalic dopamine system (an index of DA synthesis). Ro 16-6028 caused a dose-related decrease in DA turnover in mesotelencephalic DA terminal fields; at the highest dose tested (5.0 mg/kg, ip) DOPA accumulation returned to control levels in the region of the ventral tegmental area (VTA). These data suggest that the muscle relaxant properties of BDZ agonists are not essential for anxiolytic action.

The binding of a series of tritiated ligands including GBR-12935, cocaine, nomifensine, nomadine, and metabolites, to the dopamine transporter protein has been reported. These radioligands bind in a 

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**P57.10**

**Manganese Depletes Cathecolamines and Biopeter in Cultured Bovine Adrenal Chromaffin Cells. B.J. Slipetz*, O.H. Viveros and H.D. Daniels. Dept. of Pharmacology, Veterans Administration Medical Center, Durham, North Carolina.**

The primary manifestation of manganese (Mn) neurotoxicity in vivo consists of a decrease in striatal dopamine (DA) levels; several mechanisms for this effect presently are being considered, including direct displacement of DA from its storage sites by the metal ion. In an attempt to elucidate the mechanisms involved, bovine adrenal chromaffin cells in culture were used as a model adrenergic neuron. The cells were incubated with 1.5 mM Mn for 4 to 18 h, 37°C, pH 7.4. Manganese content determined over time. Studies with MnCl2 indicate that the uptake of Mn by membranes is Mn(II)-dependent and essentially complete within 5 min. Upon exposure to Mn for up to 18 h, cell Mn content had increased by 30% and by 72 h had dropped 96%. Since Mn as a cation is neurotoxic, we were interested in determining its effect on tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. Mn(II) has been shown to deplete DA and serotonin in vivo and in vitro in various species. Mn(II) exposure to bovine adrenal chromaffin cells resulted in a decrease in cell Mn content; with in 24 h had dropped 96%. Since Mn is the catalyst for tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis, and has been shown to be present in bovine chromaffin cells in cultures, we hypothesized that Mn would decrease the concentration of DA in vivo. Thus, cathecolamine losses following Mn exposure would be due to: 1) direct displacement of storage vesicles (short-term) and 2) an inhibition of DA synthesis (long-term).

**P57.11**

**IDENTIFICATION OF THE SITES ON TYROSINE HYDROXYLASE THAT ARE PHOSPHORYLATED IN INTACT PC12 CELLS. John W. Haycock.**

Department of Biochemistry & Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119.

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, is phosphorylated at multiple sites. In vitro, TH purified from rat brain homogenates has been shown to be phosphorylated at sites 8, 9, 15, 17, 19, 20, 23, 31, 32, 34, 36, 37, 38, 39, 40, and 150. In situ and in vivo, however, five tryptic phosphopeptides have been separated from TH phosphorylated in PC12 cells and rat corpus striatum (Neurosci. 14:1080). In the present studies, the tryptic phosphopeptides from TH phosphorylated in intact PC12 cells were subjected to microsequencing analysis. PC12 cells were harvested and incubated in 33P-containing buffer prior to combined treatment with veratridine, forskolin and phorbol dibutyrate. The cells were sonicated, and TH was partially purified from the high-speed supernatant by heparin-Sepharose chromatography. The eluate was dialyzed against FAGE and electrophoresed by nondenaturing electrophoresis to nitrocellulose. Excised 33P-containing bands were digested with trypsin, and the five, limit-digest phosphopeptides (PC1-PC5) were separated by reversed-phase HPLC and analyzed with a Model 477A (ABI) pulsed liquid sequencer. PC1 and PC2 phosphorylated by secretagogues, contain serine8. PC3, phosphorylated by forskolin, contains serine53. PC-4, shown by others to be phosphorylated by NGF, contains serine40. PC-3, shown by others to be phosphorylated by EGF, sequenced poorly but appears to contain serine8. PC5, phosphorylated by forskolin, contains serine40. PC-3, shown by others to be phosphorylated by NGF, sequenced poorly but appears to contain serine40.

In vitro, serine8 is phosphorylated by calcium/calcium-dependent protein kinase II (CAMK-II); serine53 can be phosphorylated by at least 4 different protein kinases; and serine40 is phosphorylated by an unknown protein kinase that copurifies with TH. The present data are consonant with the hypothesis that CAMK-II mediates the effects of secretagogues on TH phosphorylation.

**P57.12**


Bovine adrenal medullary cells, the prototype of the developing neurotoxic effect of norepinephrine to epinephrine, has been purified and characterized biochemically, immunologically and molecularly. In the present study we sought to determine biochemical and immunological characteristics of a recombinant bovine PNMT. Bovine PNMT cDNA inserted in an expression vector was used to transfet a C127 mouse fibroblast cell line. Recombinant bovine PNMT clonal cells expressing bovine PNMT and also stains immuno­ histochemically cells and processes in the cultures. Recombinant bovine PNMT has four charge isoforms (PI = 5.27, 5.07, 5.51 and 6.21) in decreasing order of relative abundance. Only the two minor charge forms are common between the recombinant and native enzymes. However, the existence of some variation in the charge isoforms between native and recombinant enzymes may result from differences in post-translational modification produced by the host cell. Our results suggest that recombinant PNMT, expressed from bovine cDNA in a mouse fibroblast cell line, is enzymatically active and shares many common features with native bovine adrenal enzyme. Supported by grant # MH 44043.

**P57.13**

**CHARACTERIZATION OF D1 Dopamine receptors in the bovine pineal gland. V. Simoesca*, L. Murin and M. Eblad.** Dept. of Pharmacology, Univ. of Ljubliana, 43d and Dewey Ave., Omaha, NE 68105.

Previous studies from our laboratory have shown that bovine pineal glands contain a high concentration of dopamine (4.0 mg/g tissue) and dopamine D2 receptors. In the present studies, the activity of serotonin N-acetyltransferase, inhibiting the basal activity at 0.1 µM and stimulating it at 100 mM in this study we examined dopamine D1 receptors in pineal gland. The assays were carried out in 2 ml of 0.05 M Tris buffer plus ions (pH 7.4) containing 150 µo g protein and 0.4 mM [3H]SCH 23390 (72.5 Ci/mmole) as ligand. Incubations were for 15 min at 37°C and were terminated by vacuum filtration followed by three 5 ml washes with ice-cold buffer. In association rate studies, equilibrium was reached within 10 min with 0.4 or 0.04 mM [3H]SCH 23390 and remained constant with time. Association rate constants (kA) were calculated to be 0.65 ± 0.01 min-1. Dissociation of [3H]SCH 23390 was rapid with a half-time of 4 min and a dissociation rate constant (kD) of 0.15 ± 0.01 min-1. Kinetic studies gave an estimated Kd of 0.23 µM. Saturation studies (using 0.01 to 3.00 mM [3H]SCH 23390) indicated the presence of only one binding site with a Kd of 0.05 ± 0.05 nM and a receptor density of 974 ± 41 fmol/mg protein, which is about 30 times the density of D2 receptors in pineal. Competition binding studies with a variety of agents active at dopamine and serotonin receptors indicated that the majority of the binding was due to D1 dopamine receptors. The results of this study indicate that the functions of dopamine D1 receptors are modulated through both D1 and D2 dopamine receptors. This study was supported in part from the grants Simone and Cino del Duca (V.S.), NS23975 (L.C.M.) and ES03949 (M.E.)

**P57.14**

**EFFECTS OF INHIBITORS OF EPINEPHRINE (E) FORMATION ON PINEAL E CONTENT. J. Opacka-Juffry*, M.C. Ruiz de Elvira* and C.W. Comn.** Dept. Anatomy & Human Biology, King’s College London, U.K.

Estimation of norepinephrine (NE) and E turnover in the pineal gland of female Wistar rats was attempted using FLA 63, an inhibitor of dopamine ß-hydroxylase. Paradoxically, 10 mg FLA 63/kg i.p. resulted in a significant increase (+77%) in pineal E content within 2 hours without affecting that of NE (-22%). The biological implications of this phenomenon remain to be elucidated. [Supported by MRC G8220475 N.]

$\textbf{517.15}$

**REGULATION OF DOPAMINE SYNTHESIS IN VIVO BY SELECTIVE D-1 RECEPTOR ANTAGONISTS. P.A. Johnson and M.F. Galloway, NFRU, Lafayette Clinic, Neuroscience Institute and Department of Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI.**

Given recent evidence for the potential involvement of 5-HT systems in reward, the present study examined the effect of a 5-HT-1A agonist, 8-OHDPAT, and a 5-HT-1B agonist, TMPP, on dopamine (DA) synthesis in vivo. DA synthesis was measured in rat DA accumulation after administration of NDS-1015 (100 ng/kg, 30 min before sacrifice). Administration of TMPP (30 nmol/kg i.c. or 30 umoles/kg s.c.) produced a four-fold increase in DA in the nucleus accumbens (NA) and striatum (ST) and a 55% increase in prefrontal cortex (PFC), whereas 8-OHDPAT (30 nmol/kg i.c.) produced only a modest increase or was ineffective. In contrast, 8-OHDPAT (30 nmol/kg) decreased DA accumulation in NA (-30%), and exerted no effect on DA synthesis in ST or PFC. However, coadministration of 8-OHDPAT and TMPP appeared to attenuate the decrease in DA produced by TMPP in the NA and ST. Following pretreatment with reserpine, TMPP did not reverse DA synthesis. In rats treated with gamma-butyrolactone (CBL), TMPP did not reverse the CBL-induced increase in DA; rather, a further enhancement (60%) of DA over CBL alone was observed in the DA-rich areas. In addition, TMPP did not block the ability of quinpirole to reverse the CBL-induced increase in DA. Thus, 5-HT-1B receptors appear to be involved in the regulation of DA synthesis in vivo. (Support: DA: 04120, MH 4227, State of Michigan DHE)

$\textbf{517.16}$

**REGULATION OF DOPAMINE RELEASE BY SEROTONIN (5HT) 1A AND 1B AGONISTS MEASURED BY MICRODIALYSIS. E. Benloucif and M. F. Galloway, Lafayette Clinic, COH Program, Dept. of Psychiatry, Wayne State Univ. Sch. Med. Detroit, MI 48002.**

Work in our laboratory indicates that agonists with specificity for the serotonin 1A and 1B receptors alter synthesis of dopamine (DA), as well as serotonin. In vivo, we have initiates a series of studies utilizing microdialysis to investigate the effects of the 5-HT agonist 5-hydroxytryptophan (5HTP) on DA release in vivo. Microdialysis probes were placed in the anterior-lateral striatum of chloral hydrate anesthetized rats and perfused with a modified Ringers solution containing the 5HT reuptake inhibitor cilorglam (1 uM) at a rate of 2.1 ml/min. Recovery was relatively stable in the second hour following probe implantation, and 30 min samples yielded an average 0.8 pg/ul of DA and 0.15 pg/ul of 5HT. Agonists were administered at least 2 hours after the completion of the MPTP treatment. Local administration of the 5HT-1B agonist TMPP (1 mM in the microdialysis probe) increased extracellular DA to an average of 7 pg/ul (9 fold increase over baseline, N=3). Extracellular 5HT was not decreased under these conditions. TMPP did not substantially affect recovery of DA or 5HT standards in vitro. Administration of the 5HT-1A agonist 8-OHDPAT (10 uM i.c. or 3 or 30 umoles/kg, i.p.) did not alter DA release. These findings support the hypothesis that 5HT-1B agonists alter dopamineergic activity. (Supported by MH-41227, DA-04120, and the State of Michigan DHE)

$\textbf{517.17}$

**COLD-SWIM STRESS INDUCES TIME-DEPENDENT CHANGES IN CENTRAL NEURAL PHENETHYLANOLINE N-METHYL TRASFERASE (PMT) AND EPINEPHRINE SYNTHESIS. E. LaMotte and L. Narasimhachari, Lafayette Clinic, Dept. of Pharmacology, The Ohio State University College of Medicine, Columbus, OH, 43210.**

Aromatic L-amino acid decarboxylase (AADC) is the second enzyme in the sequence leading to the formation of catecholamines. It is not considered to be rate limiting in the formation of hydroxyindoleacetic acid (HIAA) under L-DOPA treatment. Recently we have reported that light as well as D2 adrenergic and dopamine (DA) D-1 receptor antagonists increase enzyme activity in retina. We now provide evidence that AADC activity of the striatum can be modulated by DA receptor antagonist drugs. The ability to modulate AADC may be, in part, determined by the vitality of dopaminergic striatal nerve terminals, since we were able to increase AADC activity with drugs which do not penetrate into control animals; MPTP, 30 mg/kg, ip, was administered to mice for 7 days. This treatment resulted in a 50% decrease of DA content and AADC activity in the striatum. After three weeks post-treatment there is recovery of AADC to near control values but not of DA content. Administration of the selective D-1 receptor antagonist SCH 23990 (24h) after the completion of the MPTP treatment resulted in an increase of striatal AADC activity in a dose- and time- dependent manner. SCH 23990, 5 mg/kg, produced a maximal response in about 6 h in insolated mice but had no effect in the non-insolated. Additionally haloperidol and the selective D-2 antagonist haloperidol augmented AADC activity in the striatum of MPTP-treated mice. Again, these compounds had no effect in untreated mice. Administration of a single dose of the selective D-1 agonist SKF 38193 or the selective D-2 agonist quinpirole had no effect on enzyme activity in both control and MPTP-treated mice, i.e. [3H]5HT uptake was not decreased in the MPTP-treated mice. Since SCH 23990 prevented the SCH 23990-induced increase of AADC activity in the MPTP-treated animals. These observations may have significant implications for parkinsonian being treated with L-DOPA alone or together with a DA agonist.

$\textbf{517.18}$

**EFFECT OF ACUTE AND DAILY COCAINE AND NEUROTENSIN ON EXTRACELLULAR DOPAMINE IN THE ACCUMBENS AND A10 REGION. R.L. Kallio and P. Duffy. Dept. of VACP, Wadsworth Veterans Hospital, Pullman, WA 99164-6520.**

Acute peripheral injection of cocaine, and microinjection of serotonin (5HT) into the A10 dopamine (DA) region produces an increase in motor activity in rodents. After daily administration of either the drug the behavioral response is augmented. Enhanced DA transmission in the A10 region (NA) has been implicated in these effects. To directly evaluate a role of DA release into the NA, a removable dialysis probe (250 mm wide x 2 mm long) was inserted into the NA of conscious rats. In rats pretreated with daily cocaine (15 mg/kg, ip x 4 days) or NT (0.5 mmol/side 4 days) an acute injection of the drug produced a significantly greater increase in extracellular DA in the NA of rats not pretreated with daily saline. It has been postulated that a decrease in somatodendritic DA release in the A10 may occur after daily cocaine injection and by increasing sensory input into the A10 region, it was found that while acute cocaine elevated extracellular DA in the A10 region, after daily cocaine treatment, an acute injection no longer increased DA release. Thus, behavioral sensitization to cocaine and neurotensin is associated with an augmentation in extracellular DA in the NA, and a decrease in the A10 region.

$\textbf{517.19}$

**MODULATION OF STRIATAL AROMATIC LAMINO ACID DECARBOXYLASE, M. HadickeConstantinou, C.P. Sylvia*, P. Hubble*, L.A. Issac**, T.A. Wenegrat* and N.H. Naff. Departments of Psychiatry and of Pharmacology, The Ohio State University College of Medicine, Columbus, OH, 43210.**

Aromatic L-amino acid decarboxylase (AADC) is the second enzyme in the sequence leading to the formation of catecholamines. It is not considered to be rate limiting in the formation of hydroxyindoleacetic acid (HIAA) under L-DOPA treatment. Recently we have reported that light as well as D2 adrenergic and dopamine (DA) D-1 receptor antagonists increase enzyme activity in retina. We now provide evidence that AADC activity of the striatum can be modulated by DA receptor antagonist drugs. The ability to modulate AADC may be, in part, determined by the vitality of dopaminergic striatal nerve terminals, since we were able to increase AADC activity with drugs which do not penetrate into control animals; MPTP, 30 mg/kg, ip, was administered to mice for 7 days. This treatment resulted in a 50% decrease of DA content and AADC activity in the striatum. After three weeks post-treatment there is recovery of AADC to near control values but not of DA content. Administration of the selective D-1 receptor antagonist SCH 23990 (24h) after the completion of the MPTP treatment resulted in an increase of striatal AADC activity in a dose- and time- dependent manner. SCH 23990, 5 mg/kg, produced a maximal response in about 6 h in insolated mice but had no effect in the non-insolated. Additionally haloperidol and the selective D-2 antagonist haloperidol augmented AADC activity in the striatum of MPTP-treated mice. Again, these compounds had no effect in untreated mice. Administration of a single dose of the selective D-1 agonist SKF 38193 or the selective D-2 agonist quinpirole had no effect on enzyme activity in both control and MPTP-treated mice, i.e. [3H]5HT uptake was not decreased in the MPTP-treated mice. Since SCH 23990 prevented the SCH 23990-induced increase of AADC activity in the MPTP-treated animals. These observations may have significant implications for parkinsonian being treated with L-DOPA alone or together with a DA agonist.

$\textbf{517.20}$

**DIFFERENTIAL TETRAHYDROBIOPTERIN METABOLISM IN CENTRAL AND PERIPHERAL MONOAMINE NEURONS. G. Kapatos, C. Haegens, G. Miranda, N. Bajen and V. Konouci, Center for Cell Biology, Sinai Research Institute, Sinai Hospital of Detroit and Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI.**

Although tetrahydrobiopterin (BH4) is limiting in the synthesis of monoamines, virtually nothing is known regarding the synthesis and degradation of this essential cofactor. In addition, clinical observations suggest that BH4 metabolism may be different in central and peripheral monoaminergic neurons. Monolayer cultures of norepinephrine (NE) sympathetic (SYM) neurons derived from the neonatal rat and noradrenergic (NA) dopamine-containing (DA) neurons derived from embryonic day 15 rat brain and noradrenergic (NE) sympathetic (SYM) neurons derived from the neonatal rat superior cervical ganglia were used as models of central and peripheral monoamine neurons, respectively. Cultures were incubated with either N-acetyl-serotonin (NAS, 200uM) or 2,4-Diamino-6-hydroxypteridine (DHP, 10uM), compounds which inhibit BH4 biosynthesis by different mechanisms. Cultures were harvested at 1-8 hours and BH4 levels determined. Both NAS and DHP produced identical, and rapid declines in NE BH4 levels, exhibiting a 1/2 of 3 hours. In contrast, while DHP decreased DA BH4 levels in neurons, with 24 hours, NAS was without effect. These data suggest that BH4 metabolism is much more rapid than expected, and may be different within central DA and peripheral NA neurons. (supported by NS-26081)
518.1
REPEATED STIMULATION OF D1 Dopamine RECEPTOR ENHANCES GROWING AND NEURONAL RESPONSES TO SKF-38393 (SKF) F.D. White, X. T. Liu, and D. M. Malin; Dep. of Psychology, Neurophysiology, & Biometry, Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207

Behavioral and extracellular single unit recording techniques were employed to examine rat grooming and neuronal responses of caudate-putamen (CPU) cells, respectively, to the selective D1 agonist, SKF-38393 (SKF), following 3 weeks pretreatment (8mg/kg, bid, day, s.c.) with the same compound. After 1 week drug withdrawal, the amount of time in which rats engaged in grooming following SKF (8mg/kg) challenge was significantly prolonged as compared to either untreated or saline-treated rats. Observations of the behavioral patterns during the grooming, in conjunction with the neuronal recordings, the inhibitory responses of CPU cells to microinjections of SKF were enhanced in SKF-treated rats when compared to controls. The repeated SKF pretreatment also enhanced the inhibitory responses of CPU cells induced by the selective D2 agonist quinpirole. However, neither behavioral nor electrophysiological supersensitivities were observed in rats with SKF treatment without 1 week drug withdrawal. These results suggest that, with a period of drug withdrawal, chronic stimulation of D1 receptors with SKF may represent the receptor supersensitivity which enhances the enabling effect of D1 receptors on D2 responses (Supported by ARRA, USHR Grants DK-04093, and MT-40632 to FJW).}

518.2

Recent studies have suggested that sympathetic innervation of the spleen may directly contact cells of the immune system and thus alter the course of an immune response (Feldman et al., Ann. N.Y. Acad. Sci. 483: 81, 1985). To investigate this possibility, macrophages were isolated from rat spleen by adhesion to plastic and incubated with lipopolysaccharide (10 μg/ml) for 18h in the presence of varying doses of norepinephrine (NE). Secreted and membrane-bound samples were then tested for lymphocyte activating factors (LAF) using [3H]-thymidine incorporation into P815 (IgM) stimulated mouse thymocytes and for tumor necrosis factor (TNF) activity using L929 cells. The results show that both secreted and cell associated LAF and TNF activity are diminished in NE-treated cells. Dose response curves demonstrated a humped curve with suppression noted at 10−7 to 10−5 M NE was more potent than epinephrine and the effect could be partially reversed with propranolol but not with phentolamine, indicating the presence of a beta-receptor. Since factors released by macrophages influence many aspects of the immune response these results suggest a functional role for sympathetic innervation of the spleen in immune regulation. Supported by NS 22347 and RMS 1089.

518.3

One week of exposure to supplemental dietary calcium lowers blood pressure in weanling SHRs. The mechanism responsible for the alteration in blood pressure have not been identified. This study investigates the effects of dietary calcium on plasma catecholamines and blood pressure reactivity to bolus infusions of norepinephrine. Twenty-one day old SHRs were maintained on either a high (2.0%) or low (0.1%) calcium diet for 7 days. On the eighth day, direct arterial blood pressure was recorded followed by a 0.5 ml blood sample withdrawal or by bolus infusions of L-threo-DOPS, and 20 mg/kg norepinephrine (NE). The calcium deficient SHRs had significantly higher blood pressures than SHRs fed high calcium diets (117 mmHg vs 105 mmHg, p<.05). Plasma NE was higher in the animals on low calcium diets but the difference did not reach statistical significance (480 pg/ml low diet vs 369 pg/ml high diet, p=07). There were significant differences in blood pressure reactivity to bolus infusions of NE (p<.001) between the two diet groups with animals in the low diet group having more prolonged pressor episodes at all three dose levels. The results indicate that differences in vascular responsiveness to NE, or differences that may be responsible for the observed differences in blood pressure.

518.4
EFFECTS OF DIETARY TYRISOXIN AND OTHER AMINO ACIDS ON HEMODYNAMIC RESPONSES AND PLASMA AND TISSUE TYROSINE LEVELS. N.S. Eisenberg* and T.J. Meher. Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115.

In catecholamine (CA)-containing neurons made to fire rapidly for prolonged periods, maintained CA synthesis and release is believed to be dependent on tyrosine (TYR) availability. Sympathetic reflexes become activated in rats subjected to a continuous O2/N2/mn (hemorrhage) and in response to 20u/ug/kg i.v. hydralazine (HDZ). In these studies dietary TYR (4X the normal amount) maintained blood pressure (BP) in HDZ-induced hypotension and BP and heart rate (HR) during HEM. Diets high (4X normal) in phenylalanine (PHE), alanine (ALA), and valine (VAL), as well as 5X normal TRY failed to maintain BP or HR during severe HEM. The effect of dietary amino acid supplementation on plasma (PL), peripheral and brain tissue stores of TRY after a 5-day feeding period was also tested. PL TRY significantly increased by 54% and 77% above control levels in the 4X and 5X TRY, respectively, Both TRY diets significantly elevated heart, adrenal, kidney, spleen, brainstem and cortex TRY; while only 4X TRY increased liver TRY. TRY significantly elevated TRY only in PL and brain. ALA, which increases BP decreased TRY in PL and tissues and may be utilized when CA synthesis depends on additional TRY, such as in hypovolemic or HDZ-induced HEM.

518.5
METABOLISM OF L-THREO-3,4-DIHYDROXYPHENYLISERINE (L-THREO-DOPS) IN MOUSE BRAIN: IN VITRO AND IN VIVO STUDIES. I. Libert, L. H. F. Ten Dam, and A. J. G. Koolkamp (ISPON; E.Housepian). Dep of Neurology, Coll. of Med, Lafayette Clinic, Detroit, MI 48207

L-threo-DOPS is the stereoisomer of DOPS (L-3,4-DHPS) in mouse brain. In vitro and in vivo studies, using an in vivo test apparatus containing a dish of wet mash and either a food pellet or wood block, were performed to observe the effects of the D2 agonist quinpirole. When CA synthesis depends on additional TRY, such as in hypovolemic or HDZ-induced HEM.

518.6

Weanling (21 day old) Sprague-Dawley rat pups were s.c. injected with 0 (saline), 0.05, 0.1, 0.5, or 1.0 mg/kg/3cc of the D2 agonist quinpirole and individually placed in a test apparatus containing a dish of wet mash and either a food pellet or wood block. Behaviors were recorded for 5 min. via time-sampling at 30 and 60 min. post-injection. The 3 highest doses of quinpirole increased the amount of wall climbing/supporting behavior, forward locomotion, and forward locomotion. These doses of quinpirole also induced both non-directed chewing behavior (munching) as well as chewing directed at the wood pellet suggesting that the chewing was not strongly nutritive in nature. Although in adult animals quinpirole has been reported to induce dose-dependent changes in locomotion, sniffing and rearing, oral behaviors in adults are not induced by quinpirole alone (Arnt et al., 1987). and indeed have been observed to be inhibited by hypovolemic treatment (Eliison et al., 1988).

Supported by DA0478 to LFS.
D-1 Dopamine (DA) Receptor Stimulation Induces Glycogen Phosphorylase in Rat Striatum Slices. L.D. Kremer*, C.P. Saller and R.J. Salemes (SPON: G. Dubinsky). Dep. of Pharmacology, ICI Pharmaceuticals Group, Wilmington, DE 19897.

D-1 DA receptor stimulation increases GMP formation. Since glycogen phosphorylase is activated via a GMP-dependent process, the possible role of D-1 receptor stimulation in glycogen phosphorylase activation was examined in homogenized rat striatal slices. Incubation of tissue with a selective D-1 agonist, SKF 38393 (50 and 100 µM), activated glycogen phosphorylase. The SKF 38393-induced activation was prevented by pre-incubation with the D-1 selective antagonist, SCH 23390 (10 µM). By itself, SCH 23390 had either no effect or slightly decreased striatal glycogen phosphorylase activity. Therefore, D-1 receptor stimulation appears to activate glycogen phosphorylase in vitro, indicating a possible role for DA in the regulation of brain carbohydrate metabolism. In fact, preliminary in vivo studies provide some support for this possibility. However, the data obtained thus far, while often significant, have been variable, suggesting that other factors such as endogenous dopaminergic activity or stress might be important factors in vivo.

Alpha-1 Adrenergic Mediation of Noradrenaline-Stimulated Glycogenolysis in the Rat Olfactory Bulb. M. Hugon, D. Coopersmith, and M. Leonard. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

The rat olfactory bulb has very high levels of glycogen phosphorylase, the enzyme which breaks down glycogen to glucose (Coopersmith, R. and Leon, M. J. Comp. Neurol., 261:148, 1987). Noradrenaline is a potent glycogenolytic agent in olfactory bulb slices, causing activating phosphorylation of glycogen at concentrations three orders of magnitude lower than the dose required in mouse cortical slices. We now report that phenylephrine, an alpha-1 adrenoceptor agonist, is a potent glycogenolytic agent in olfactory bulb slices as is noradrenaline. Olfactory bulb slices were incubated with tritiated glucose for 30 min. to label glycogen. Phenylephrine, at 1 µM to 10 µM, was then added to the slices. After 25 min, slices were homogenized, radioactive glucose and amino acids were removed, and the radioactivity remaining in glycogen was determined. Phenylephrine induced a concentration-dependent breakdown of glycogen. The maximal effect was a 55% loss of label, the same as seen with noradrenaline. The EC50 was approximately 100 µM, slightly lower than that for noradrenaline. Clonidine, an alpha-2 agonist, was ineffective at a dose of 10 µM. In cortex, noradrenaline-stimulated glycogenolysis is mediated by beta-adrenoceptors, and alpha agonists have little effect. The contrasting results in olfactory bulb are consistent with the high density of alpha-adrenoceptors found in this structure.

Continuous and Intermittent Administration of SKF 38393 or Quinpirole Differentially Affect D-1 and D-2 agonist-induced rotational behavior induced by these selective agonists were compared in rats with a unilateral 6-hydroxydopamine lesion of the median forebrain bundle. Rats were divided into 3 treatment groups: 1) control vehicle + intermittent vehicle (control group), 2) continuous agonist + intermittent vehicle, or c) continuous vehicle + intermittent agonist. Continuous treatments were given via Alzet osmotic pumps (Model 2ML2) implanted. Intermittent treatments consisted of i.p. injections given once daily. The same daily dose was administered for both continuous and intermittent agonist treatments. SKF 38393 was given at a dose of 12.5 mg/kg/day in a vehicle of 12.5% ascorbate in 50% DMSO; quinpirole was given at a dose of 1 mg/kg/day in 0.1% ascorbate in water. Following 19 days of treatment and a 3 day washout, rats (n=6 in each group) were tested for rotational response to either SKF 38393 (1.25 mg/kg, i.p.) or quinpirole (0.1 mg/kg, i.p.). Continuous treatments abolished the subsequent rotational response to SKF 38393 (0.9% of control) but had no effect on the response to quinpirole. Intermittent SKF 38393 substantially reduced the rotational response to SKF 38393 (9% of control), but increased the response to quinpirole (175% of control). Continuous treatments had no effect on the rotational response to either SKF 38393 or quinpirole. Intermittent quinpirole greatly enhanced the rotational response to both SKF 38393 (279% of control) and quinpirole (202% of control). These data suggest that the D-1 D-2 dopamine agonists differentially affect rotational behavior mediated by D-1 and D-2 dopamine receptors and, furthermore, that continuous and intermittent treatment schedules can profoundly influence the nature of the subsequent rotational response.


In vivo and in vitro studies suggest that dopaminergic neurons of the A10 area inhibit the firing of prefrontal cortex (PFC) neurons through the activation of GABAergic interneurons. We have previously investigated the effects of D1 and D2 agonists and antagonists on the electrophysiologically-evoked release of [3H]-GABA in PFC slices (30). In the present study, we have found that this D1 agonist-induced release of [3H]-GABA was largely calcium dependent. It was not affected by the D1 agonist SKF 38393 but inhibited by the two D2 agonists haloperidol (0.2 mg/kg i.p.) or (+)-sulpiride (10 mg/kg i.p.) and, more importantly, by a D1 selective agonist, SCH 33390 (0.2 mg/kg i.p.). The D2 agonist LY 171555 remained ineffective. The effects of 10-5M RU 24926 were fully reversed, and those of 10-4M RU 24926 were only partially reversed by the selective D2 antagonist sulpiride 10-5M. Although exogenous DA (10-5M) was devoid of effect, amphetamine (10-5M) inhibited it. Hence, D-2 mediated release in this effect was antagonized by 10-5M sulpiride. Our results show that in vivo, the activation of DA receptors with D2 preferring agonists produces: 1. Thierry et al., 1986, Brain Res. Bull., 16:155-160 2. Pient-Soria et al., 1987, Brain Res., 425:263-274. Supported by DRET grant 88/1193.
518.13

**DOPAMINE IN CAT VISUAL CORTEX FOLLOWING AN ACTICHELINESTERASE AGENT. A.T. Townsend* and A.H. Kirby (SPON: A. Porouch. USAARL, F.D. Box 577, Ft. Rucker, AL 36362.**

Administration of diisopropylfluorophosphate (DPF), an irreversible inhibitor of acetylcholinesterase (AChE), results in a marked reduction in the cat visual evoked response (VER), and biochemical changes in visual cortex within 1-2 hours. After 20-24 hours, the VER recovers. However, AChE activity does not. These experiments were done to investigate the role of dopamine (DA) in these changes.

After i.v. DFP, anesthetized and paralyzed cats show a preferential transient frequency loss in the VER (14 of 14) and increased DA turnover in visual cortex (13 of 14) at all survival times, even when the VER had recovered to baseline.

DFP was applied directly to the surface of the visual cortex of anesthetized cats to determine if DA turnover is locally mediated. Preliminary results show increased DA turnover in DFP cortex compared to control (opposite side, same animal). It appears that increased DA turnover in cat visual cortex is locally mediated after DFP, and may play a critical role in VER changes.

518.14

**COMPUTATIONAL MODELING OF α- AND β- NORAEDREGIC ACTIONS AT DENDRITIC VS. SOMATIC LOCUS OF DENDATE GRANULE NEURONS. D.J. Zigmund* and P.K. Staggan (spoon: E.S. Goldstein). University of Pennsylvania, Philadelphia, PA 19104 and Albert Einstein College of Medicine, Bronx, NY 10461.**

Noradrenergic (NE) is a modulator of neuronal plasticity in the hippocampal dentate gyrus. The mechanism of NE's action is not well understood. In the present study, NE was examined for effects on the field potential evoked by perforant path synapses and those direct long-lasting actions on granule cell membranes. There are multiple effects on NE receptors mediated by both α- and β-receptor subtypes, and on both somatic and dendritic sites of action. A balance of effects determines whether firing is enhanced or reduced, and various cell types can be differentially modulated by NE.

In light of difficulties determining somatic vs. dendritic sites of action experimentally, the authors studied the effects of NE on granule neurons and simulated effects of specifically activating single α- or β-receptor actions on conductances at either somatic or dendritic loci. Using a comprehensive model of the dentate gyrus, they were able to investigate the role of somatic vs. dendritic sites of NE action, suggesting that spatial distribution of noradrenergic receptors mediates a sensitivity of postsynaptically altering NE regulation of signal processing and storage.

518.15

**TURNOVER OF BIOMICRONE AMINES IN DISCRETE AREAS IN THE RAT BRAIN AND THE SIGNIFICANCE OF L-AMINIC ACID DECARBOXYLATING ACTIVITIES. A.Y.C Shum*, D-J Juang*, C-F Chen* and J-Y Wang. Department of Pharmacology, National Yang-Ming University Medical College and Department of Physiology, National Defence Medical Center, Taipei, Taiwan, ROC.**

Accumulation of the intermediates dihydroxyphenylalanine (DOPA) and 5-hydroxytryptamine (5HT) following l-aminic acid decarboxylase (LAAD) inhibition with the drug α-methyltyrosine (MST) permits the simultaneous estimation of turnover of the neurotransmitters catecholamines (CA) and 5-hydroxytryptamine (5HT). However, the nature and significance of this inhibition remains controversial. The present experiments were designed to clarify some of these questions. In vivo enzymic analyses following in vivo inhibition of NSD do not produce depletion of tissue levels by itself, the accumulation of these amines following inhibition of the metabolizing enzyme monoamine oxidase (MAO) was significantly reduced. Exogenous administration of DOPA and 5HT in conjunction with MAO inhibition resulted in accumulation of 5HT and mainly dopamine (DA) in all areas studied. It is thus concluded that LAAD was effectively and dose-dependently inhibited by NSD and that DOPA accumulation mainly represented DA turnover. The lack of effects on tissue levels of monoamines may be due to the fact that NSD also appeared to inhibit MAO.

518.16

**THE EFFECTS OF STRESSOR CONTROLLABILITY ON REGIONAL CHANGES IN MESOCORTICOLIMBIC DOPAMINE ACTIVITY. L.W. Fitzgerald*, R.W. Keller, S.D. Glick, J.N. Carlson. Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208.**

While it has been known for some time that stress may induce an activation of dopamine (DA) neurons projecting to the medial prefrontal cortex (PFC) and the nucleus accumbens (NA), in the rat, few studies have assessed the effects of the ability to control the stressor on these measures. Rats exposed to a series of uncontrollable but not controllable footshocks develop a transient behavioral deficit upon later shock escape testing. It was of interest to examine the effects of stressor control on changes in DA activity in the terminal regions of the mesocorticicolimbic DA system. Male rats were exposed to a series of mild escapable footshocks (ESC), identical inescapable footshocks (INS) or no footshock (CTL) in a yoked triadic design. Within 1 hr of footshock rats were decapitated, and bilateral samples of the PFC, NA and striatum were removed and assayed for DA, DOPAC and HVA. INS induced a bilateral depletion of DA, a large right > left difference in DA utilization (DOPAC/DA) in the PFC and a depletion of DA on the left side of the NA. ESC induced a bilateral activation of DA utilization in the PFC and a left > right difference in DA utilization in the NA. PFC/DA was bilaterally elevated above controls in NA for both the INS and ESC groups. Additional studies have examined these measures at 24hr following footshock. The results indicate that differences in susceptibility to behavioral deficits caused by lack of stressor control may depend upon lateralized brain function (Support ES04032 J.N.C.)

519.1

**VASOPRESSIN (AVP) RECEPTOR REGULATION FOLLOWING THE SENSITIZATION OF THE RAT BRAIN WITH REPEATED AVP ADMINISTRATION. P. Poulin and R.S. Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta Canada T2N 4N1.**

Vasopressin (AVP) administered into the ventral septal area (VSA) of rats produces severe motor disturbances which result from an interaction of AVP with V1 type of AVP receptors and involve a sensitization process whereby a first activation (sensitization) of AVP causes minor motor disturbances, while a second dose, two days later causes severe motor disturbances. Since repeated AVP injections result in the sensitization of AVP receptors at different time intervals, we found that the sensitization of the rat brain to AVP induced motor disturbances is time dependent and occurs only if the AVP is administered at different time intervals and lasting for 4 days. If animals were repeatedly challenged with AVP on 6 consecutive days, no tolerance was observed. In binding studies with [3H]AVP as the ligand, Scatchard analysis revealed no differences in Kd or Bmax in VSA membranes from sensitized or control animals (Kd: 1.2 vs. 0.9 nM; Bmax: 28 vs. 21 fmol/mg protein, respectively). Thus the mechanism by which such sensitization process takes place is time dependent and does not appear to be via an alteration of AVP receptors affinity or numbers.

519.2

**CHRONIC THEOPHYLLINE EFFECTS ON ADRENERGIC A1 RECEPTOR BINDING AND IN-VITO ELECTROPHYSIOLOGY IN THE HIPPOCAMPUS. G.R. JUHÁS, M.F. JANVIER AND R.F. BERMAN. Wayne State University, Detroit, MI; Ustra-Leyp Corp., Summit, N.J.**

Chronic treatment with theophylline (THEO) results in the up-regulation of adrenergic blocking A1 receptors in the hippocampus. In vivo binding studies in homogenates of hippocampi from the same subjects. Sixty-four male rats received either 100 mg/kg/day of theophylline or a vehicle for 14d. 48hr following the last injection 0.4mM hippocampal slices were prepared. Field potentials were recorded from stratum radiatum, hilus and CA1 for the degree of inhibition of this response in adenosine compared across chronic theophylline groups. A2 Receptor binding assays using 3H-CHA (0.1-30nM) revealed a significant 20% increase in 3H-CHA binding (p<.01) without a change in Kd. THEO treatment also resulted in an increased sensitivity of the field response to 1.2 and 5.0, but not 25uM adenosine (p<.05). These results suggest that chronic adenosine receptor antagonist increases a multi-compartmental A1 receptor which supports the idea that this change in receptor number mediates an increased sensitivity to exogenously applied adenosine.

Recently Ashton et al. (Epilepsy Res. 2:65, 1988) have shown that solufzazine, a water soluble nucleoside transport inhibitor, may act by increasing extracellular adenosine (ADO) levels in the CNS. We have investigated the effect of prolonged treatment (14 days) with solufzazine (200µM/hr; Le; Alzet minipumps) on locomotor activity and [3H]-ADO receptor densities in male Sprague-Dawley rats. ADO-A1 receptor binding was measured by Scatchard analysis of [3H]-R-PIA binding, while ADO-A2 receptor binding was estimated by the method of Yeung and Green (Nauny-Schmiedeberg's Arch Pharmacol, 325:218, 1984). Solufzazine significantly decreased locomotor activity by 38% (p=0.0097) during the last 24 hours of drug treatment. At the same time, radioligand binding to ADO-A2 receptors was significantly increased by 41% (p=0.0344), while no decrease in ADO-A1 binding was noted in corpus, hippocampus, cerebellum, or striatum. Previously, we have reported desensitization of ADO-A2 receptors in the striatum following prolonged treatment with ADO analogs (Porter et al., JPET, 244:218, 1988). Our present results further strengthen the hypothesis that solufzazine causes its effects through increases in endogenous ADO.

519.5 EFFECT OF CORTICOSTERONE ON VIP RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM AND PITUITARY. A. Sarrieau*, M. Dussaillant* and W.H. Rostene (SPON: L. Quentin). INSERM U55158 rue du Faud St-Antoine 75012 Paris France.

Previous reports showed that the limbic system and the hypothalamo-pituitary axis contain VIP and glucocorticoid receptors. In these structures, corticosteroids increase or decrease the endogenous level of VIP in the pituitary and the hippocampus, respectively. However, the effect of glucocorticoids on VIP receptors has never been studied.

Regulation of VIP binding sites by corticosterone (CORT) was investigated in the hypothalamo-pituitary axis. The CORT, and the hippocampus of 1) control, 2) adrenalectomized (ADX), 3) ADX and CORT-implanted, 4) non ADX and CORT-implanted rats or for a week.

125I VIP specific binding was determined by 20µm frontal sections incubated in vitro and processed for autoradiography. Our results indicate a hypercorticism-induced decrease of VIP receptor density in the subiculum and CA3 of the Ammon's horn, a hyporcorticism-induced increase in the supraoptic, periventricular and arcuate nuclei; CA2, CA4 subfields of the hippocampus and in the adenosinopexis; no variation was detected in the suprachiasmatic nucleus and the dentate gyrus. Our data demonstrate a possible influence of peripheral glucocorticoid hormones on central and pituitary VIP receptors.

519.6 OXYTOCIN PEPTIDE FRAGMENTS AS NEUROMODULATORS: REVERSAL OF UPREGULATION OF PRE- & POST-SYNAPTIC D2 Dopaminergic Receptors. B. Fields*, K. Starg, Dept. of Pharmacology, Univ. Ill. Coll. Med., Chicago, Ill. 60612 and Clinical Research, Upjohn Co. Kalamazoo. We have previously reported that prolonged treatment with diazepam, a benzodiazepine (BZ) with long plasma half-life, attenuates radioligand binding to adenosine (ADO) A2 receptors (Hawkins et al., Neuropharmac., 27:1131, 1988). Now we have examined the effects of prolonged treatment (10-20 days) with triazolam (0.5, 1, 2mg/kg/day; 3B, a BZ with short plasma half-life) on ADO-A2 receptor binding. ADO-A1 receptor binding was measured by Scatchard analysis of [3H]-R-PIA binding while A2 receptor binding was estimated by the method of Yeung and Green (Nauny-Schmiedeberg's Arch Pharmacol, 325:218, 1984). After 10 days, none of the doses of triazolam altered the number of A1 receptors in all brain areas studied. However, A2 receptor binding was significantly increased by 31% (p=0.003) in striatal membranes from 2mg triazolam-treated rats. In contrast, the dose of 0.5mg sham had no significant effect on [3H]-A2 binding to the A2 receptor by 15% (p=0.003). Plasma concentrations for the doses of 0.5 and 2mg/kg/day were 0.38±0.07 and 2.33±0.77 ng/ml, respectively. After 20 days of treatment none of the doses of triazolam altered radioligand binding to either A1 or A2 receptors. These results suggest that ADO-A2 receptors may play a role in the CNS actions of BZ. Supported by a Grant from the Upjohn Co.


Acute injection of amphetamine (AMPH; 9.2 mg/kg, i.p.) in combination with 10.0 mg/kg, i.p. (pindolol) produces a long-lasting depletion of brain dopamine (DA). We used the same doses and evaluated three treatment regimens: 1) AMPH + pindolol for their effects on DA-mediated behaviors. Male F344 rats were tested for spontaneous locomotor activity 1 wk or 3 wk after the conclusion of drug treatment, and for apomorphine (APD)-induced stereotypy (repetitive, non-exploratory sniffing) at 10 days. At 1 wk, animals injected 1x or 3x with AMPH + pindolol showed suppressed horizontal and vertical activity during the first 5 min of testing. By 3 wk, the 3x group had recovered to control activity levels. APD-induced stereotypy was increased in rats injected 1x or 3x with AMPH. The decrease in activity may be related to the AMPH-induced depletion of DA. The increase in APD-induced stereotypy suggests that the recovery of activity may be associated with an up-regulation of post-synaptic DA receptors. (Supported in part by the VA; NIH MHS-26449; Tourette Syndrome Assoc., and the Scottish Rite Schizophrenia Research Program, N.J., U.S.A.)

519.8 COMPARISON OF LONG-TERM EFFECTS OF LITHIUM AND CARBAMAZEPINE ON PHOSPHOINOSTIDE TURNOVER IN CULTURED CEREBELLAR GRANULE CELLS. X.-M. Gao and D.-M. Chuang (Spon: D. Kirsch), Lab. of Preclinical Pharmacol., NIMH, S. Elizabeth Hospital, Washington, DC 20037.

The mechanisms of action of lithium (Li·) and carbamazepine (CBZ) for the treatment of manic depression are still unclear. We have studied the effects of Li· and CBZ on basal and muscarinic cholinergic receptor-mediated phosphoinositide turnover in cerebellar granule cells. 3-Day exposure of cultured cells to Li· affected biphasically basal and carbachol-stimulated [3H]inositol monophosphate accumulation. At low concentrations (i.e., 10 µM) of Li·, basal and carbachol-induced PI turnover was enhanced; at higher concentrations (10-50 mM), these activities were markedly attenuated. The inhibition of carbachol response observed with 20 mM Li· was associated with the maximal stimulation. The enhancement induced by 2 mM Li· was time-dependent with activities progressively increased between day 2 and 7. 3-Day exposure of granule cells to CBZ inhibited basal and CBZ-stimulated PI turnover; however, higher concentrations (50-100 µM) of CBZ induced a marked attenuation of carbachol-induced PI breakdown. This inhibition of carbachol response was reversed by longer-term (7-day) exposure to CBZ. Thus, Li· and CBZ appear to have complex but distinct effects on PI turnover in cerebellar granule cells.
519.9  
ETOPERIDONE, A NOVEL ANTIDEPRESSANT, MAY DOWN-REGULATE HUMAN PLATELET IMPRIMARE RECEPTORS.  
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House Rd.  
Nonclassical antidepressants are thought to bind to imipramine receptors (IR's), although the newest antidepressant agents have not been well characterized in this regard. Changes in Bmax of the IR in 17 depressed patients randomized to treatmet with etoperidone (ETO) or placebo (PLA). Platelet membranes were harvested prior to and at 6 and 14 weeks of treatment. The IR was defined by binding of [3H]- 
imipramine in the presence of 15 micromolar desipramine. IR Bmax declined in both groups (for ETO: 642.3, 470.4, and 408.1 fmoles/ml platelet membrane protein; for PLA: 657.4, 426.3 and 521.4 at baseline, 6 and 14 weeks). Comparison of IR Bmax with scores of the Hamilton Depression Scale (HAM-D) using multivariate repeated-measures analysis indicated that at a given level of antidepressant response, IR Bmax in the ETO group was significantly lower than in the PLA group (Lambda=.52, F(2,10)=4.54, p=.05). These results suggest that ETO may downregulate platelet IR's in parallel with its antidepressant efficacy.  

519.11  
DIFFERENTIAL EFFECTS OF VARIOUS ANTIDEPRESSANT TREATMENTS ON M-CPP-INDUCED DECREASES IN FOOD INTAKE IN FAWN-HOODED RATS.  
LCS, National Institute of Mental Health, Bethesda, MD 20892.  
We recently reported that the Fawn-Hooded (FH) rat strain is functionally subsensitive to m-chlorophenylpiperazine (m-CPP, a 5-HT,  
agonist)-induced decreases in food intake (Psychopharmacology 94: 588-592,  
1988) and locomotor activity (Pharmacology, Biochemistry, Behavior 49: 867-871,  
1990), and that chronic administration of clorgyline (a selective MAO type A inhibiting  
agonist)-induced decreases in food intake were significantly attenuated  
(M-CPP) in the FH strain. We hypothesized that these effects would be accentuated by m-CPP treatment accompanying other behavioral alterations characteristic of FH rats.  

519.12  
ANTIDEPRESSANT-INDUCED DOWN REGULATION OF CENTRAL \( \beta_1 \) ADRENOCEPTORS: REGIONALLY SELECTIVE EFFECTS.  
To investigate receptor down-regulation in normal tissue, we measured the down-regulation in the FH strain using a competitive antagonist IR (ERI). We also determined whether regional differences exist in the degree of down-regulation of \( \beta_1 \) adrenoceptors in normal tissue and in tissue altered by the administration of antidepressants which affect central \( \beta_1 \) adrenoceptors.  

519.13  
FINANCED YAWNING RESPONSE TO THE DOPAMINE D-2 AGONIST, 
LY717555, IN ADULT RATS THAT WERE TREATED IN DEVELOPMENT 
WITH LY717555.  
LCS, National Institute of Mental Health, Bethesda, MD 20892.  
Because dopamine D1 and D2 receptor antagonists,  
after development of striatal D1 and D2 receptors, respectively, we determined whether the D1 and D2 agonists (SKF38393, LY717555) and antagonists (SCH23390, spiroperidol), respectively, would alter the yawning response to LY717555 in rats that were treated in development with the above agents. Rats were treated once a day for 12 consecutive days from birth with SCH23390 (0.3 mg/kg/d), spiroperidol (0.3 mg/kg/d), SKF  
(3.0 mg/kg/d), or LY 717555 (3.0 mg/kg/d) or with or without 6-OHDA (6-OHDA; 6-OHDA) and were challenged with LY717555 (50 ug ip) at 2 weeks of age. The incidence of yawning was increased in the LY717555 group. Treatment in development with spiroperidol did not alter the yawning response of the SCH23390 group. Treatment in development with SKF38393, SCH23390, and control groups. The SCH23390 groups, with or without 6-OHDA, gave the same response, which was greater than the control group. These findings indicate that treatment only with a D2 agonist in development will enhance the D2 mediated yawning response in rats that are studied in adulthood.  

519.14  
ENHANCED STEREOTYPIC RESPONSES TO DOPAMINE AGONISTS IN 
ADULT RATS THAT WERE TREATED IN DEVELOPMENT WITH 
SKF38393.  
R.B. Kostrzewa and A. Handi, East Tennessee State  
University, Quinlan-Disher College of Medicine,  
Johnson City, TN 37614.  
Because the dopamine D1 receptor antagonist, SCH23390, alters development of striatal D1 receptors, we studied how developmental treatment with a D1 agonist would affect responses in adulthood. Rats were treated once a day for 12 days from birth with SKF38393 (3.0 mg/kg/d,  
i.p.), and were challenged with the D1 agonist at 6 to 12 weeks of age. SKF38393 increased locomotor activity, as well as the incidence of digging, gnawing, and rearing. Rats in the SKF38393 group that were given an additional neonatal treatment with 6-hydroxydopamine (133 ug, iat  
200), had enhanced digging, gnawing, and rearing responses. The SKF38393 group that were given an additional neonatal treatment with 6-hydroxydopamine (133 ug,  
iat 180), had enhanced digging, gnawing, and rearing responses. These findings indicate that D1 agonist treatment of developing rats will significantly increase the subsequent challenge dose of the agonist. The new animal models provide additional means of studying the dopamine receptor system. (Supported by BMB 2 DOT805959)
Mittel NERVOUS SYSTEM PHYSIOLOGY. Tyrone Lee and M. Noella Picarril.*. Psychopharmacology Unit, E.J. Kenton, B.C. Perry, and J. Kellar. Deps. of Pharmacology, George Washington University, Washington, DC 20037 & Georgetown Univ., Washington, DC 20007

Cocaine abuse is currently regarded as one of the major threats to our societal infrastructure. We have demonstrated long-term changes in functional activity of dopaminergic brain regions following perinatal cocaine exposure in the rat. The present study examines the effects of perinatal cocaine exposure on the development of dopaminergic receptors. Rats were administered cocaine, 50 mg/kg sc, each day between day 1 and 10. On postnatal day 60-70, central dopamine receptors were identified with [3H]SKF 38393, a ligand with high selectivity for the D1 receptor, using autoradiography. Treated rats showed an increase in ligand binding in a number of cerebral structures including those of the limbic and motor systems compared to vehicle injected controls. Many of these changes were previously shown to have increased metabolic activity in adulthood following perinatal exposure to cocaine. These data suggest that cocaine exposure during pregnancy may be responsible for long term functional alterations in the dopaminergic system in brain.

Supported by NIDA Grant # DA14188.

DIFERENTIAL REGULATION OF ALPHA-1 ADRENERGIC RECEPTOR SUBTYPES BY RESERPINE. E.L. Grinnell, J.A. Blundell,* L. West-Johnson, R.C. Perry, and J. Kellar. Deps. of Pharmacology, George Washington University, Washington, DC 20037 & Georgetown Univ., Washington, DC 20007

ALTERATIONS IN THE BINDING CHARACTERISTICS OF GLUCOCORTICOIDS IN OBESE ZUCKER RATS. B.D. White* and R.J. Martin. Deps. of Foods and Nutrition, University of Georgia, Athens, GA 30602.

Oseizue rats have been shown to lack a circadian rhythm of plasma corticosterone. Elevated morning concentrations of plasma corticosterone in obese rats result in relatively high corticosterone concentration throughout the 24-hour day. The purpose of this study was to characterize glucocorticoid receptors in brain regions known to be involved in negative feedback to determine if receptor alterations may be involved in the lack of a corticosterone rhythm in obese rats. Eight obese and ten male Zucker rats (3 mo. old) were adrenalectomized and sacrificed within 24 hours. The brains were removed and the anterior pituitary, hypothalamus, and hippocampus isolated. Liver tissue was taken. Tissues were homogenized in a Tris buffer and the cytosolic fractions were collected by ultracentrifugation. Radioactive assays were performed using [3H]dexamethasone (0.1-30nM). Non-specific binding was determined by the addition of 1000-fold excess of cold dexamethasone. The KD of binding was higher in the anterior pituitary of obese rats than in lean rats. No other phenotypic difference in binding was observed in the brain regions. Liver tissue from obese rats showed both a higher KD and a lower maximal binding as compared to lean rats. Lower maximal binding in the liver of obese rats may reflect down-regulation of glucocorticoid receptors by higher daily concentrations of plasma corticosterone. A higher KD in the anterior pituitary suggests a lower sensitivity to glucocorticoids. Thus, it is possible that obese Zucker rats have less feedback inhibition at the level of the anterior pituitary.

CORRECTION OF AUTOGRAPHIC TRITIUM QUENCHING. B.M. Samson and A.W. Toga. Dept. of Neurology, UCLA, Los Angeles, CA 90025

Accurate quantification of autoradiograms generated by initiated probes is hampered by the phenomenon of differential tissue quenching. Gray and white matter containing equivalent amounts of tritium will generate different autoradiographic signals because white matter absorbs more of the low energy beta-emissions than gray matter. These experiments have investigated an autoradiographic method for the detection and correction of tissue quenching.

Our method involves the autoradiography of blocks of tritium-imregnated plastic overlaid with thin unlabeled brain sections. Gray and white matter efficiencies varied with tissue thickness. Despite this difficulty, overlay images still can provide a means of digital quench correction for autoradiographic data obtained from adjacent sections.

519.15

NERVOUS SYSTEM PHYSIOLOGY. Tyrone Lee and M. Noella Picarril.*. Psychopharmacology Unit, E.J. Kenton, B.C. Perry, and J. Kellar. Deps. of Pharmacology, George Washington University, Washington, DC 20037 & Georgetown Univ., Washington, DC 20007

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Our method involves the autoradiography of blocks of tritium-imregnated plastic overlaid with thin unlabeled brain sections. Gray and white matter efficiencies varied with tissue thickness. Despite this difficulty, overlay images still can provide a means of digital quench correction for autoradiographic data obtained from adjacent sections.
Chronic verapamil treatment affects imipramine binding to human platelets. M. Rehavi*. A Cohen" N. Eider, M. Carmi and A. Weizman*. Dept. of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel.-(SPON: N. Dascal)

Several recent reports have suggested that calcium channel blockers, mainly verapamil, are effective in the treatment of affective disorders. Verapamil was found to be a competitive inhibitor of both [H]imipramine binding and [H]serotonin uptake to rat brain and human platelets. The authors found that calcium channel blockers, nitrendipine and diltiazem were inactive at the serotonin transporter site. Antidepressants inhibited ([H]desmethoxyverapamil binding (Ki=2.11-1.07µM) but did not affect [H]nitrendipine binding to rat cerebral cortex membranes. Increased density of [H]imipramine binding sites (24%, p<0.055) was detected in platelets of carriers of chronic (at least two months verapamil treatment (80-360 mg/day) when compared to normal healthy age and sex-matched controls. This effect did not affect the affinity of imipramine to its binding site. Modulatory effect of in vivo chronic verapamil treatment on the serotonin transporter is suggested.

DOPAMINE INCREASES THE OPENING FREQUENCY OF KAINATE-SENSITIVE ION CHANNELS IN WHITE PERCH HORIZONTAL CELLS.


Dopamine enhances responses of cultured white perch horizontal cells to kainate and L-glutamate (Knapp and Dowling, Nature 325, 437). We have used whole-cell current noise analysis and single-channel recordings to determine those properties of the excitatory amino acid-gated channels (i.e. conductance, number, and open time) that change following exposure of the neurons to dopamine. When the levels of kainate or L-glutamate were raised or lowered around voltage-clamped horizontal cells, calcium channel blockers, nitrendipine and diltiazem were inactive at the serotonin transporter site. Antidepressants inhibited ([H]desmethoxyverapamil binding (Ki=2.11-1.07µM) but did not affect [H]nitrendipine binding to rat cerebral cortex membranes. Increased density of [H]imipramine binding sites (24%, p<0.055) was detected in platelets of carriers of chronic (at least two months verapamil treatment (80-360 mg/day) when compared to normal healthy age and sex-matched controls. This effect did not affect the affinity of imipramine to its binding site. Modulatory effect of in vivo chronic verapamil treatment on the serotonin transporter is suggested.

SECOND MESSENGERS: ADENYLALE CYCLASE

INTERACTIONS BETWEEN ADENYLALE CYCLASE AND PROSTAGLANDIN D2 SIGNALING SYSTEMS IN NCB-20 CELLS. C.L. Boyajian and D.R. Cooper*. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Individual signal transduction systems within cells appear to interact at a number of levels. We have been studying the interplay between Ca2+-mobilizing and cAMP-generating systems. In most brain regions, Ca2+ concentrations corresponding to those achieved upon stimulation of the PI system, stimulate adenylate cyclase activity via calmodulin. Recently, we have shown that the Ca2+-concentrations elicit a profound (45%) inhibition of the plasma membrane adenylate cyclase activity of NCB-20 cells (a neuroblastoma x hamster hybrid cell line). This effect is entirely independent of calmodulin, yet highly cooperative (nH=67) for Ca2+ ions. It requires a stimulated activity state of the cyclase, and is not sensitive to the actions of pertussis toxin. In intact NCB-20 cells, bradykinin, which stimulates PI hydrolysis and Ca2+-mobilization, causes a significant inhibition of cAMP production, which is not due to any stimulation of cAMP phosphodiesterase activity. These data indicate that considerable crosstalk occurs between Ca2+-mobilizing and cAMP-generating systems, which may vary across cell types in accommodating specific physiological demands.

EFFECTS OF CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE ON RAT SPINAL DORSAL HORN NEURONS IN VITRO. R. Cerna*, G. Carbe*, L. Kangrige and M. Bread* (SPON: W. G. Vandeven). Dept. of Vet. Pharmacol. and Physiol., Iowa State University, Ames, IA 50011, USA.

Intracellular recordings were made from rat spinal dorsal horn neurons in the in vitro slice preparation to study the actions of cyclic 3',5'-adenosine monophosphate (cyclic AMP) on the membrane permeability analogue of cyclic AMP. 8-Brcyclic AMP produced a slow depolarization of the resting membrane potential, an increase in membrane input resistance and excitability. Activation of the adenosine adenylate cyclase of dorsal horn neurons by forskolin, or the phosphodiesterase inhibition by methylxanthines, resulted in a slower depolarization accompanied by an increase in spontaneous synaptic activity and enhancement of dorsal root-evoked EPSPs. In the presence of TTX, 8-Brcyclic AMP and forskolin enhanced, in a reversible manner, the depolarizing responses of a proportion of dorsal horn neurons to [H]NMDA (200 µM) which increased the mean current but changed neither the mean vs. variance relationship nor the power spectrum of the agonist-induced currents, suggesting an increase in channel open probability but not in mean channel open time. In single-channel recordings from cell-attached patches with kainate or L-glutamate in the pipette, dopamine approximately doubled the frequency of 5-10 pS channel openings, without altering the size of the unitary events, and only slightly increasing the mean channel open time. These results suggest that dopaminergic enhancement of responses to excitatory amino acids in horizontal cells is mediated predominantly by an increase in the frequency of channel opening.

DI RECEPTOR COMPLEX SUPERSENSITIVITY RESULTS IN A DECREASED INHIBITORY RESPONSE OF ADENYLALE CYCLASE.

M.G. De Monteis, P. Devoto*, A. Porcella*, P. Sabat* and A. Tagliamonte*. Inst. of Pharmacology and Biochem. Pathology, University of Cagliari, Italy.

Rats chronically exposed to SCH 23390, a selective D1 dopamine (DA) receptor blocker, show increased number of striatal DA binding sites (-32%) and increased adenylyl cyclase response to DA stimulation. DA superresponsivity was also observed in the striatum of the enzyme, since both DA and forskolin stimulation produced a marked increase of the Vmax. In these rats the inhibition of DA-stimulated adenylyl cyclase activity by opiates (DADLE and Dynorphin-1-13) was the same in control rats and in rats chronically exposed to SCH 23390. On the other hand, the increased Vmax of adenylyl cyclase produced by long-term D1 receptor blockade was accompanied by a significantly decreased response of basal enzyme activity to the inhibitory effect of opiates and muscarinic agonists. We conclude that the endogenous inhibitory tonus on adenylyl cyclase activity is increased after chronic SCH 23390 treatment.
TUBULIN STIMULATES ADENYLYL CYCLASE IN C6 GLIOMA MEMBRANE VIA TRANSFER OF GUANINE NUCLEOTIDE FROM TUBULIN TO ADENYLYL CYCLASE. G. S. Dhilon and M. L. Koenig. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington D.C. 20307.

A number of peptides and physiologically active substances have been reported to affect the neuronal activity and release of neurotransmitters by affording second messenger systems (cAMP-dependent and/or calcium-dependent mechanisms). In the present study we investigated the effects of a number of synthetic and natural peptides on adenylate cyclase activity in synaptosomes prepared from rat cerebral cortex.

Synaptosomes were prepared from freshly dissected rat brain cortex by the method of Booth and Clark (Biochem. J., 176:365-370, 1978). Adenylate cyclase activity was measured in P$_2$ fractions by the method of Solomon et al. (Analytical Biochem., 58:141-148, 1974). CRH (1 uM), VIP (10 uM) and NE (50 nM) increased basal adenylate cyclase activity by 30%, 118% and 79% respectively. The effects of CCRP, NPY and PTH (1 uM) were less pronounced, producing increases in adenylate cyclase activity of 34%, 16% and 19% respectively, Somatostatin (1 uM) and isoproteranol (50 uM) had no effects on the enzyme activity. Vasopressin (1 uM) alone or in combination with CRH did not affect the adenylate cyclase activity under the same condition. These results indicate that adenylate cyclase activity could be modulated by neurotransmitters and that the effect of a given peptide may be dependent on the type of receptor involved.

Ongoing investigation of DA signal transduction in the amygdaled complex focuses on the inhibitory influence of $\alpha$-adrenergic receptors on cyclic AMP efflux from spinal cord neurons by using $\alpha$-adrenergic agonists and antagonists. This research will provide new insights into the role of cyclic AMP in the regulation of neuronal activity in the spinal cord.
520.11

WIN 40989 (Pravadinol), an aminoalkylindole (AAI) analog, together with structurally related compounds devoid of cyclansetamine activity, were previously shown to inhibit both basal and forskolin-stimulated adenylyl cyclase (AC) activity in rat cerebral membranes. This inhibition was GTP dependent, suggesting that AAI analogs act through the GTP-binding G protein and that forskolin-stimulated AC activity is mediated by this G protein. In isolated cerebral membranes, AAI agonists exhibited only partial additivity of AC inhibition with the AAI agonist batclifen and the adenosine A1 agonist N6-(2-

520.12
PHARMACOLOGICAL CONCENTRATIONS OF MELOTIN INHIBIT ADENYLYL CYCLASE ACTIVITY IN RAT BRAIN MEMBRANES. F. Hashemi and I.F. Miles (SFP: E.S. Hershey), Dept. Biomedical Sciences, Pennsylvania State University, 1200 Main Street West, Hamilton, Ontario, L8N 3C5

Preincubation of rat cortical membranes with high concentrations of melatonin (10-1000 µM) for 1 hour at 30°C caused an inhibition of forskolin-stimulated AC activity with an EC50 of about 200 µM. A maximum inhibition of about 30% was produced by 750 µM melatonin. Various receptor antagonists including metapromone, phenolamine and the central Gi receptor antagonist, Ro25-1788, did not block the effect of melatonin. Similarly, the inhibitory effect of dexamethasone was not blocked by Ro25-1788 suggesting the involvement of peripheral rather than central Gi receptors. Since melatonin also binds to peripheral Gi receptor sites, its pharmacological effects on AC activity may involve these sites. (Supported by the Ontario Mental Health Foundation and MRC, Canada.)

520.13
REVERSAL OF 8-BROMO-CAMP (8-B-CAMP) AND NEUROTENSIN (NT)-INDUCED ATTENUATION OF DA INHIBITION OF DA NEURONS BY A PROTEIN KINASE (PK) A INHIBITOR, H8. W. X. Shi and B.S. Bunney, Deps. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We have previously reported that 8-b-cAMP, forskolin and IBMX mimic the action of NT, i.e., attenuate DA-induced inhibition of DA neurons. When administered concomitantly, IBMX potentiates NT's effects while SQ255536, an adenylyl cyclase inhibitor, attenuates the effect of NT. These results suggest that NT may produce its effect by increasing intracellular cAMP, which in turn activates PKA. In order to determine whether PKA is involved in the action of NT, we have employed an inhibitor of PKA, H8. The spontaneous activity of DA cells was recorded extracellularly in brain slices. Both application of H8 (80µM, for 20-30 mins) significantly or completely blocked the modulatory effects of both 8-b-cAMP and NT. Occasionally, H8 alone potentiated DA-induced inhibition. As H8 inhibits both PK A and C, the above effect may have due to its effect on PK C. This possibility was tested by using H7, an analogue of H8, which is a more potent inhibitor of PK C than H8. In our preparation, treatment with H7 was found to be much less effective or to have no effect on NT's actions. These results not only suggest that cAMP-dependent PK C may regulate the response of DA cells to autoreceptor stimulation but also support the hypothesis that they may be involved in NT action.

520.14
PHORBOL ESTER INDUCED EFFECTS ON CAMP FORMATION IN A RAT HIPPOCAMPAL CELL LINE. Kelly A. Berg', W. W. Clark, K. L. Raft and M. C. Taylor, Dep. of Pharmacology and Physiology, Mount Sinai School of Medicine, CUNY, NY, NY 10029, and Dep. of Biology and Brain Science, MIT, Cambridge, MA 02139.

Initial steps of hormone-elicited signal transduction in excitable cells may be modulated by intracellular events that are initiated by activation of a second messenger system. For example, protein kinase C (PKC) mediated sensitization of adenylate cyclase (AC) may be one aspect of modulation between the phosho-inositol and AC signaling systems. We are studying the effects of activation of PKC on forskolin (FRSK) and receptor-mediated cAMP production in a novel, neuronal cell line (HT4) derived from embryonic rat hippocampus. Simultaneous application (30 mins) of phorbol 12-myristate 13-acetate (PMA, 1 µM), with either isoprenaline (ISO, 0.1 µM); beta-adrenoceptor agonist), 3-N-ethylcarboxamidoadenosine (NECA, 1 µM); Adenosine A1 receptor agonist) or FRSK (1 µM) amplified the formation of cAMP 3-4 fold over FRSK or receptor stimulated cAMP formation alone (table) as determined by RIA. In addition, preliminary studies suggest that proteinphlbin (alpha) adrenergic) and 5-HT (serotonergic) also enhance FRSK stimulation of cAMP in this cell line.

520.15

The binding of the cardioselective muscarinic antagonist AF-DX 116 ([112](dihydroxyethyl)methyl)-L-piperidinyl]acetate)-5,11- dihydro-12-pyridine (1:1 [benzene]:ethanol) was compared with its ability to interfere with muscarinic receptor- mediated inhibition of adenylyl cyclase activity in the longitudinal muscle of the rat ileum. When measured by the competitive inhibition of the binding of the muscarinic antagonist [3H]-methylscopolamine ([3H]MNS), the binding properties of AF-DX 116 were consistent with a two site model in which 72% of the receptors exhibited high affinity. The dissociation constants were high and the low affinity sites (Kd1 and Kd2) were estimated to be 0.078 and 2.3 µM, respectively. The highly efficacious muscarinic agonist oxotremorine-M caused a concentration-dependent inhibition of adenylyl cyclase activity in homogenates of the longitudinal muscle of the ileum, with the maximal inhibition and the concentration of oxotremorine-M causing half-maximal inhibition (EC50) being 31% and 0.46 µM, respectively. When measured by competitive antagonism of the adenylyl cyclase response, the dissociation constant of AF-DX 116 (Kd) was estimated to be 0.1-17 µM. Thus, there was good agreement between the Kd values of AF-DX 116 indicating that it is primarily the high affinity AF-DX 116 site which couples to adenylyl cyclase in the longitudinal muscle of the rat ileum. Supported by NIH Grant N26511.

520.16

Previously, we found that the firing of noradrenergic neurons of the locus coeruleus (LC) was increased by agents that elevate cAMP levels (forskolin and Ro20-1724) or mimic cAMP action (e.g., 8-Br-cAMP). Here, we investigated whether the activation induced by above agents in LC neurons could be blocked by an inhibitor of cAMP-dependent protein kinase (PKI, Walsh inhibitor). LC neurons in rat brain slices were recorded intracellularly with blunt electrodes (10-30 MΩ) in S-MOPS (340 mM) buffered with 20 mM sodium carbonate (pH 7.4). With neither PKI (100µg/ml; rabbit muscle, Sigma), PKI was ejected into neurons by passing negative current (0.6-2.0 nA) for 15-25 min. Portosion with 8-Br-cAMP (1 mM), forskolin (10 µM) or Ro20-1724 (100 µM) for 10 min increased the firing rate of LC neurons by ~2 fold when control electrodes were used. When PKI was ejected into LT neurons, the activation induced by 8-Br-cAMP, forskolin, or Ro20-1724 was attenuated by ~75%. After recovery, recovery was accelerated by PKI ejection and some cells hyperpolarized after discontinuing the cAMP-active agents. When a second dose of the above agents was administered following recovery, the increase in firing rate was further reduced or completely abolished by PKI.

Since the increase of LC cell firing produced by the cAMP-active agents was attenuated or blocked by PKI, our results suggest that this increase is mediated by a cAMP-dependent protein kinase.
EVIDENCE FOR AN ENDOGENOUS INHIBITOR OF 3H-FORSKOLIN BINDING IN BRAIN. D. R. Gehlert, Experimental Therapeutic Branch, NINDS, NIH, Bethesda, MD 20892.

The compound forskolin is a potent activator of adenylyl cyclase which is believed to activate via a direct interaction with the catalytic subunit. Binding studies using 3H-forskolin have indicated that forskolin binding in the brain is stimulated by the interaction of Gsp(THP) and Gα with βα and the binding sites are found primarily in the basal ganglia. In order to determine if the catalytic unit of adenylyl cyclase is regulated via the forskolin binding site in vivo, the present study was initiated to determine if an endogenous factor may interact with the forskolin binding site.

Rat brains were homogenized in an acidic solution and extracted twice with ether. The resulting solution was concentrated using vacuum centrifugation and pumped onto a semipreparative C-18 column and eluted with an acetonitrile gradient. Fractions which inhibited 3H-forskolin binding to rat brain membranes were rechromatographed using additional reverse phase steps and the final active fraction separated using gel filtration chromatography. The inhibitory activity appeared to be proteins in the 30-50 KDa molecular weight range. The inhibition of binding was dose dependent and susceptible to protease digestion.

These results indicate that the binding of forskolin can be regulated by endogenous proteins in the brain.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CYTOSKELETON


Spinal cord of the teleost Apteronotus albifrons, unlike mammalian cord, can regenerate axons and produce new neurons after injury. This study characterizes the precursors cells and initial neuronal differentiation in vitro. Putative precursor cells were cultured from the caudalmost tip of regenerating spinal cords (L-15 medium with 10% fetal calf serum, on polylysine-coated plastic). After 7 days in vitro, the caudalmost cells are flat and polygonal or fibroblastic. They do not exhibit thin, neurite-like extensions, and do not stain with monoclonal antibody against neurofilaments. After 14 days in vitro, cells in the caudalmost cultures begin to show altered morphologies, having more rounded somas and thin, spiky projections 5-100 µm long. Small lamellar areas at the tips of these projections may represent early growth cones. After 18 days in vitro, some of these cells stain with the anti-neurofilament antibody. These results suggest that 1. cells from the caudalmost spinal cord can undergo differentiation over time in vitro, and 2. changes in morphology may precede the production of neuron-specific filaments during differentiation in vitro. Supported by NS-25951 and the College of Veterinary Medicine and Biomedical Sciences, Colorado State University.

201.2 ALtered MAP2 DISTRIBUTION IN THE CYTOPLASM OF PURKINJE CELLS AND CA1 PYRAMIDAL CELLS OF THE REELER MUTANT MOUSE. P.R. Patriotio and R.S. Nowakowski. Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School and Physiology/Neurobiology Program, Rutgers University, Piscataway, NJ 08854.

The distributions of microtubule associated proteins (MAP1 and MAP2) in the cerebellum and hippocampus of reeler (r/o=r/o=r/o) mice were studied by using indirect immunohistochemistry with monoclonal antibodies. MAP2 is localized in the dendrites but not the soma of every normally positioned Purkinje cell of the reeler cerebellum as well as in control littermates and C57BL/6J. In contrast, MAP2 is localized in both the soma and dendrites of the CA1 pyramidal cells adjacent to the stratum radiatum and of all CA1 pyramidal cells of control littermates and C57BL/6J. In contrast, in the apical dendrites of the CA1 pyramidal cells adjacent to the stratum oriens MAP2 is decreased or absent. No differences in the cytoplasmic localization of MAP1 were observed.

Thus, in reeler mutants subsets of the Purkinje cells and the CA1 pyramidal cells have an altered MAP2 distribution. The fact that there is an increase in MAP2 in the soma of some of the ectopic Purkinje cells but a decrease in MAP2 in the apical dendrites of the CA1 pyramidal cells closest the stratum oriens indicates that the changes in MAP2 distribution are not a direct effect of the reeler mutation. Supported by NIH (NS23647) and the Schizophrenia Research Program.

IMMUNOCYTOCHEMICAL STUDY OF HUMAN LENS EPITHELIUM. S. Murayama*, T. W. Bouldin*, & K. Suzuki. Dept of Pathology, Univ. Of North Carolina, Chapel Hill, NC, 27599-7525

Therapeutic Branch, NINDS, NIH, Bethesda, MD 20892.

The developmental and aging changes of the lens epithelium in humans from 14 weeks gestation to 81 years old were studied immunocytochemically in formalin-fixed paraffin-embedded postmortem material. Antibodies employed are anti-vimentin monoclonal antibody (Osborn, 1984) and the anti-ubiquitin monoclonal antibody (Mori et al, 1987). Our study characterizes the precuror cells and initial neuronal differentiation in vitro, the caudalmost cells are flat and polygonal or fibroblastic. They do not exhibit thin, neurite-like extensions, and do not stain with monoclonal antibody against neurofilaments. After 14 days in vitro, cells in the caudalmost cultures begin to show altered morphologies, having more rounded somas and thin, spiky projections 5-100 µm long. Small lamellar areas at the tips of these projections may represent early growth cones. After 18 days in vitro, some of these cells stain with the anti-neurofilament antibody. These results suggest that 1. cells from the caudalmost spinal cord can undergo differentiation over time in vitro, and 2. changes in morphology may precede the production of neuron-specific filaments during differentiation in vitro. Supported by NS-25951 and the College of Veterinary Medicine and Biomedical Sciences, Colorado State University.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CYTOSKELETON


The distributions of microtubule associated proteins (MAP1 and MAP2) in the cerebellum and hippocampus of reeler (r/o=r/o=r/o) mice were studied by using indirect immunohistochemistry with monoclonal antibodies. MAP2 is localized in the dendrites but not the soma of every normally positioned Purkinje cell of the reeler cerebellum as well as in control littermates and C57BL/6J. In contrast, MAP2 is localized in both the soma and dendrites of the CA1 pyramidal cells adjacent to the stratum radiatum and of all CA1 pyramidal cells of control littermates and C57BL/6J. In contrast, in the apical dendrites of the CA1 pyramidal cells adjacent to the stratum oriens MAP2 is decreased or absent. No differences in the cytoplasmic localization of MAP1 were observed.

Thus, in reeler mutants subsets of the Purkinje cells and the CA1 pyramidal cells have an altered MAP2 distribution. The fact that there is an increase in MAP2 in the soma of some of the ectopic Purkinje cells but a decrease in MAP2 in the apical dendrites of the CA1 pyramidal cells closest the stratum oriens indicates that the changes in MAP2 distribution are not a direct effect of the reeler mutation. Supported by NIH (NS23647) and the Schizophrenia Research Program.


The Rat.401 protein is a 200 Kd protein specifically found in neuronal stem cells in the rat CNS, but absent from adult CNS. Outside the developing CNS we find expression only in developing skeletal muscle. Analysis of cDNA clones corresponding to the N-terminus of the Rat.401 protein identifies regions that are 30-50% homologous to intermediate filament genes in strong, neurofilament and vimentin at the amino acid level. These findings stimulated an investigation of the intracellular distribution of the Rat.401 protein with respect to other cytoskeletal components. Immunostaining of cells from the CNS precursor cell line ST15A reveals that the Rat.401 protein does not co-localize with vimentin, nor with actin or tubulin. The intracellular distribution of Rat.401 protein is unaffected by treatment of the cells with colchicine and cytochalasin B. Supported by grants from NIH, the Pew Foundation, the Rita Allen Foundation and an EMBO Fellowship to U.L.

A novel condition-dependent per Km and NMDA receptor expression in Zucker rats. Therefore, this study investigated the circadian rhythm of Zucker rats, with a focus on the role of the hypothalamus in the control of voluntary activity, using a combination of pharmacological and physiological methods. The results suggest that the circadian rhythm of Zucker rats is controlled by the hypothalamus, and that the hypothalamic clock is reset by the presence of food. This finding has implications for the understanding of the role of the hypothalamus in the control of voluntary activity and the circadian rhythm of Zucker rats.


The cDNA coding for the type III intermediate filament protein (IFP) peripherin (p75) was cloned in a NZB/KS hybrid embryo of age E11.5. The expression of the p75 gene was examined in the developing nervous system of the rat embryo using in situ hybridization. The p75 gene is expressed in the developing retina, brain, and spinal cord, with a peak expression in the retina at E13.5. The expression of the p75 gene is regulated by a variety of factors, including retinoic acid, which is known to induce the expression of the p75 gene in the retina.


Microtubule-associated protein 2 (MAP2) has been proposed to play a role in the formation of dendrites and maintenance of neuronal shape. This study examined whether developmental changes of the Purkinje cell dendritic trees could be monitored using an antibody against MAP2. Sections from the cerebellum of developing rat pups from postnatal day 1 (PD 1) to PD 12 were processed for immunohistochemistry with an antibody against MAP2. The results show that MAP2 staining is present at PD 1 and increases through PD 12, with a peak at PD 7. The staining is localized to the proximal region of the Purkinje cell dendritic tree, indicating that MAP2 plays a role in the maintenance of the dendritic tree.


A novel condition-dependent per Km and NMDA receptor expression in Zucker rats. Therefore, this study investigated the circadian rhythm of Zucker rats, with a focus on the role of the hypothalamus in the control of voluntary activity, using a combination of pharmacological and physiological methods. The results suggest that the circadian rhythm of Zucker rats is controlled by the hypothalamus, and that the hypothalamic clock is reset by the presence of food. This finding has implications for the understanding of the role of the hypothalamus in the control of voluntary activity and the circadian rhythm of Zucker rats.
522.3

SEX DIFFERENCES IN THE PATTERN OF LIPID DEPLETION FROM ADIPOSE TISSUE IN SHORT DAY-EXPOSED SIBERIAN HAMSTERS. T. L. Barrentes, Dept. of Psychology & Biology, Georgia State University, Atlanta, GA 30303.

Siberian hamsters exposed to short days decrease their body weight, an effect reflected nearly exclusively as a decrease in carcass lipid. We previously reported that internally-located white adipose tissue (WAT) fat pads (e.g., retroperitoneal WAT (RPWAT)) were depleted of lipid at a faster rate than externally-located WAT pads (e.g., inguinal subcutaneous WAT (IWAT)) following 6 or 12 wk of exposure to short days in male hamsters. In the present experiment, female and male hamsters were housed in long days (LD 16:8) or transferred to short days (LD 8:16) for 6 wk. Short day-exposed hamsters of both sexes had decreased body weight and carcass lipid content; however, relative to their long day controls, short day-housed male hamsters had greater depletions of lipid from RPWAT than IWAT, and this was reflected as a disproportionate decrease in IWAT fat cell size, with no effect on fat cell number (FCN). In contrast, female hamsters had uniform decreases in IWAT and RPWAT mass, reflected as uniform decreases in fat cell size, without affecting FCN, relative to their long day controls. These results suggest that gonadal steroids may differentially modify the responses of adipocytes to changes in daylength in Siberian hamsters in a fat pad-specific manner. Supported by NIH DK-28054.

522.4


Syrian hamsters housed under SP conditions increase their food intake (FI) and body weight (BW). To determine possible vagus nerve involvement in these responses to SP, we examined FI and BW in groups of vagotomized (VAGX; n=10) and sham operated (SHAMS; n=8) hamsters maintained under a long photoperiod (LP; L:D 14:10) or a SP (L:D 8:16). Three days after surgery, hamsters were provided a high fat diet (lard & chow) and housed under an assigned photoperiod. All animals gained weight upon the availability of the high fat diet. However, FI and BW gains were greater in SHAMS maintained under SP than in SHAMS housed under LP. FI and BW gains in VAGX hamsters maintained under LP and SP were not different than in SHAMS housed under LP. Estrous cycling during the 7th-10th week of the study was evident (from vaginal discharges) in all SHAMS and VAGX hamsters in LP, but not in any of their counterparts in SP. Our results indicate that the vagus is not important for the development of dietary obesity, but is critical for the expression of elevated FI and BW under SP. Our findings with VAGX also suggest that separate mechanisms underly seasonal changes in BW regulation and reproductive function. We are now examining whether VAGX inhibition of SP-induced hyperphagia and obesity results from blockade of vagally-mediated hyperinsulinemic responses to SP.

522.5


Siberian hamsters undergo morphological changes that are controlled by day length. Testes undergo regression, uterine weight, and total body weight are reduced in short day lengths. Hamsters do not, however, respond to short day lengths in the expected manner. They have been exposed to one extended long day (33 h of continuous light) at weaning (Spears et al., in prep.). We tested whether a 4 h extension of the light phase on the day of weaning might be mediated by phase shifting of circadian rhythms to the SD photoperiod. To test this hypothesis, animals gestated and lactated in SD were weaned at 18 days and given either an additional SD (controls) or an extended LD (experimental). Thereafter all hamsters were maintained in constant darkness. On day 35 experimental animals had significantly heavier testes (214.2 ± 31 vs 20.8 ± 10.5 ± 34, p<0.05) and body weight (255 ± 68 vs 158 ± 48 mg) days of age). Uteri were heavier in experimental than control females at 30 but not 35 days of age. An increase in photoperiod duration at the time of weaning exerts long term effects on the reproductive axis. Supported by NIH Grant HD 02982.

522.6


Siberian hamsters given a single long day (LD) at weaning and housed thereafter in short days (SD) have large gonads at day 35. The photostimulatory effect induced by one LD at weaning might be mediated by phase shifting of circadian rhythms and dependent on subsequent changes in entrainment to SD photoperiod. To test this hypothesis, animals gestated and lactated in SD were weaned at 18 days and given either an additional SD (controls) or an extended LD (experiments). Thereafter all hamsters were maintained in constant darkness. On day 35 experimental animals had significantly heavier testes (214.2 ± 31 vs 20.8 ± 3 mg) and uteri (19.4 ± 2 vs 10.5 ± 4 mg) than did controls. Because animals in constant darkness were not entrained to a SD light dark cycle, changes in phase angles of entrainment of circadian rhythms to the SD photoperiod are not necessary and are not likely to be responsible for the original phenomenon. Supported by GRANT HD-02982.

522.7

TIME MELATONIN INFUSIONS INDUCE UTERINE REGRESSION IN PINEALECTOMIZED SYRIAN HAMSTERS. M. H. Brown and G. N. Wade. Dept. of Psychology, University of California, Berkeley, CA 94720.

Pinealectomized Syrian hamsters were maintained in a 1LD:1SD photoperiod through 18 days of age. On day 18 the light period was extended by 4 h for the experimental group only. On day 19 experimental and control hamsters were transferred to a short photoperiod (8:16). Experimental males had significantly heavier gonads than control males at 30 (480 ± 22 vs 306 ± 44 mg) and 35 (553 ± 68 vs 158 ± 48 mg) days of age. Uteri were heavier in experimental than control females at 30 but not 35 days of age. An increase in photoperiod duration at the time of weaning has long term photostimulatory effects on the reproductive system. Supported by NIH Grant HD 02982.

522.8

THE PARAVENTRICULAR NUCLEI (PVN) INFLUENCE DAILY TORPOR IN SIBERIAN HAMSTERS. N. E. Ruby and I. Zucker. Dept. of Psychology, University of California, Berkeley, CA 94720.

The role of the PVN in mediating daily torpor was studied in adult hamsters maintained in a short photoperiod (8 h light/day). Animals with radiofrequency transmitters were maintained in 17°C and body temperature (Tb) recorded at 10 minute intervals using a telemetry system beginning 8 weeks after initial short day exposure. Animals manifesting at least 4 torpor bouts (Tb < 30°C) in a 14 day interval received lesions of the PVN (PVNv) or sham lesions; hamsters with neural damage outside the PVN constituted a separate group. Ablation of the PVN completely terminated the expression of torpor in 60% of animals tested, and had no effect in the remaining PVN+ hamsters. Torpor was unaffected in sham-operated animals and in those with partial damage to the PVN and other neural structures. Ablation of the suprachiasmatic nuclei (SCN) terminates the expression of torpor while pinealectomy does not. Thus, elimination of torpor in PVN+ hamsters is not due to denervation of the pineal gland. These results suggest SCN efferents that course through the PVN, or the PVN itself may be part of a neural substrate for expression of torpor.

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Anorexia nervosa and bulimia nervosa are psychiatric illnesses which manifest prolonged or permanent disturbances in eating and hypothalamic function. Evidence for cyclical abnormalities in endocrine, sleep, and thermoregulatory rhythms have been reported. We attempted to describe patterns in oral temperature (OT) rhythmicity that may reveal functional or hypothalamic deficits in eating disorder (ED) patients. We report the occurrence of chronic hyperthermia and describe cyclical patterns of OT in 25 patients. A 4-week interval of different times of day 13 patients showed chronic hyperthermia (OT>36.7°C) or unstable OT. Most patients showed hyperreactivity to ambient cold and to a cold pressor test (CPT), masked changes in plasma AVP and NE when in the cold, extreme variations in OT (2°C) at room temperature, irregular or displaced diurnal OT cycles, and a variety of differences in chronobiologic cosinor parameters (acrophase, amplitude, and mesor). Unstable OT correlated with CPT-induced heat release (r=0.71) and hyperthermia (r=0.76).

Persistence of abnormal diurnal thermoregulation rhythmicity may result from an underlying neuroendocrine imbalance in ED patients following self-starvation/malnutrition.


Bulbectomized rodents provide an animal model for agitated depression (Lumia et al. Soc. Neurosci. Abst., Vol. 14, p. 908, 1988; Jesberger, J.A. and Richardson, J.S., Behav. Neurosci. 100:256-274, 1986). We have previously shown that bilateral olfactory bulbectomy (OBX) alters the period, phase and amplitude of circadian rhythms in mice (Lumia et al., 1988) and rats (Lumia et al., Soc. Neurosci. Abst., Vol. 12, 1987) similar to changes reported for humans with agitated depressive states (Teicher et al., Arch. Gen. Psych. 45:913-917, 1998). Here we show that OBX mice have an altered daily rhythm for temperature in a 12:12 LD cycle, and a higher mean temperature (36.29 +/- 0.32 for OBX mice vs. 34.95 +/- 0.40 in controls). The controls show a typical nocturnal peak temperature which drops back to the 24-hour mean at the end of the dark period and reaches a low point for the cycle one hour after the lights go on. The OBX mice show a similar peak, but remain elevated for two hours into the light period and reach a low point three hours later than controls.


Intensity and precision of circadian wheel-running patterns were evaluated in 3 commonly available inbred rat strains: golden-Evans hooded (LE), Wistar (W), and Sprague-Dawley (SD). Intensity of wheel-running (wheel revolutions per day) significantly differed among strains under both entrained conditions (P<8.9, with LE>W>SD) and in constant darkness (P<14.7, with LE≈W≈SD). Without data on endogenous free running in constant darkness (P<0.17). In LE, initial studies of phase-delays in response to 180° phase shift (5°) white light pulses showed the expected dependence on age of magnitude of phase-delay on light irradiance (96±35' mean ± s.d.) 0.46 umW/cm² 0.06 umW/cm² 0.005 cm² 0.005 cm² p<0.05). While there was good correlation (r=0.71) between magnitude of phase-delays after exposure of the same animals to 2 identical bright light pulses at a 3-week interval, the second phase-delay was significantly larger than the first (135±24' vs. 115±37', p<0.05). A smaller delay was used in interpreting results based on exposure of the same animal to multiple light pulses, at least in this paradigm.
A FUNCTIONAL RUNNING WHEEL IS NOT NECESSARY FOR THE DEVELOPMENT OF SPLIT LOCOMOTION IN SYRIAN HAMSTERS (MESOCYTIS SYRIENSIS). R. C. H. HANCOCK AND C. E. MCCANN. The Chicago Medical School, North Chicago, IL 60064.

The fact that in continuous light (LL) the locomotor rhythm of the Syrian hamster contains two distinct components to which briefly displays a different period (tau), comprises evidence that the circadian oscillator is composed of multiple coupled oscillators. However, since rotation of the running wheel by the hamster rat, in itself, alter the phase of the locomotor rhythm (Nature 320: 198 (1986)), it is possible that "splitting" in LL represents a wheel-induced artifact rather than a manifestation of multiple oscillators. Therefore, the present experiments test whether the hamster's locomotor rhythm is induced by two rhythms, with different phases, present in the hamsters in response to "pulses" of a running wheel. Hamsters housed in constant light (in cages with a running wheel) were given a new cage once every 2 weeks at 1 of 8 different circadian time points. Half of the cages contained a wheel that was removed after 1 hour and half of the cages contained no wheel. The phase response curve (PRC) derived from the wheel "pulses" has a shape similar to the PRC generated by injections of Tz. Phase advances in the activity rhythm occur at CT's 6 and 9, and delays occur at CT's 0 and 3. The only significant phase shifts induced by transfer to new cages without wheels were phase delays at CT 0. Although presentations of new cages with or without wheels resulted in increased activity 97% of the time, no significant correlation was found within a group between the increase in activity and the magnitude of the phase shift. These results indicate that presentation of a novel stimulus which increases activity can affect the circadian clock in a less similar to that observed for the protein. DARPP-32 mRNA was also detected in the olfactory tubercle and nucleus accumbens on P0, and its development in these two areas proceeded with a similar time course as in the CP. The failure of reliably obtainable DARPP-32 mRNA prior to birth indicates that the levels are below the sensitivity of the technique.
523.5

DEVELOPMENTAL REGULATION OF HUMAN BRAIN THYMOSIN BETA-10 & ITS MODULATION IN NEUROBLASTOMA CELLS BY RETINOIDS. Alan K. Ball, James Hempstead2* and James I. Morgan2*, (Spon:T.W. Lyon).

(1) Section of Urology, Department of Surgery, UMDNJ-Newark, New Jersey 07103-2757,(2)Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.

We used high performance liquid chromatography to identify a peptide which was enriched in rat embryonic and neonatal brain tissue, but absent in the adult CNS. The peptide was found to be thymosin beta-10. (Ziai et al., 1989). Comparison of HPLC chromatograms obtained from adult (90 year old) and fetal (49 days post-conception) human brain tissue indicated that thymosin beta-10 is a major peptide species. Retinoic acid induced an inhibition of thymosin beta-10 mRNA expression in retina cells thus affecting the in vitro situation. Hence, these observations suggest that the brain is a target for retinoids.

523.7
cDNA CLONING OF RETINA COGNIN, AN EMBRYONIC CHICK CELL RECOGNITION PROTEIN. A.S.M. Krishna Rao and R.E. Hausman, Department of Biology, Boston University, 2 Cummings St, Boston, MA 02215 USA.

mRNA was prepared from eight day embryonic chick neural retina tissue and used to make cDNA. Expression libraries were constructed in lambda GT vectors. These were screened with the specific polyclonal antiserum against retina cognin. Six clones were positive for both these techniques. Multiple screening yielded five clones positive for both these techniques. These clones are currently being investigated for their retina cognin specificity by dot blotting and comparing their temporal patterns of expression during embryonic development with the known pattern of retina cognin expression. The inserts are also being cloned into plasmid vectors for sequencing and the construction of anti-sense RNA to be used for interference with cognin expression during retina development and with the differentiation of retinal neurons in vitro. (Supported by NIH grant EY04461 to R.E.H.)

523.8


The developmental appearance of gap junction mRNAs was examined during postnatal development of the rac and mouse brain. Two cDNA probes specific for the heart-type gap junction connexin32, and one specific for the neural-type gap junction connexin43, were used to probe Northern blots of total RNA isolated from the forebrain and hindbrain of rats and mice at different pre- and postnatal times. Prior to day 10, connexin32 mRNA is not detectable by Northern analysis. By days 10-15, connexin32 mRNA is evident. This mRNA appears in the hindbrain several days prior to its appearance in the forebrain. The level of connexin32 mRNA increases steadily to day 30, subsequently decreasing by the adult stage. In contrast, connexin43 mRNA is readily detectable as early as embryonic day 14. Postnatally, there is a steady increase in the level of connexin43 mRNA to day 30, followed by a slight decrease in the adult. These data suggest that there is heterogeneity in the gap junctions in brain, and that the mRNAs for two of these gap junction proteins are differentially regulated during neural development.

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523.9


P19, a cytoplasmic protein that undergoes hormonally regulated phosphorylation in neuroendocrine tumor cells, was previously purified from bovine brain. Its cDNA was recently cloned and sequenced, revealing that the gene encoding p19 has been highly conserved during mammalian evolution and is related to the gene encoding S6K. A protein expressed in embryonic and perinatal neurons using immunoblotting it was previously shown that expression of p19 is restricted to brain in adults but changes during brain development. We describe here the profile of appearance of p19 in rat brain. Using antibodies against the crosslinking agent NHS, we identified a major specific crosslinking species of 100,000 MW, identical to the one found in PC12 cells. The highest level of crosslinked receptor was observed in chicken retina cells in chicken brain. In human brain, NGF receptor was detected in the retina and in the different brain regions (telencephalon, diencephalon, mesencephalon and metencephalon). Receptor binding activity is identical to the pattern of steady state levels of receptor mRNA, suggesting that transcription of the gene can be directly correlated with the appearance of the receptor protein.

523.10

REGULATED EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR MOLECULES IN BRAIN MORPHOGENESIS. E. Forand* and M. Chan* (SPON: I. Black). Dept. of Cells, Biol and Anatomy, Cornell University Medical College, New York, NY 10021.

Expression of the NGF receptor gene is tightly regulated during chikten brain development. We describe here the profile of appearance of NGF receptor protein during chicken development. Using antibodies against the crosslinking agent EDC, we identified a major specific crosslinking species of 100,000 MW, identical to the one found in PC12 cells. The highest level of crosslinked receptor was observed in chicken retina cells in chicken brain. In human brain, NGF receptor was detected in the retina and in the different brain regions (telencephalon, diencephalon, mesencephalon and metencephalon). Receptor binding activity is identical to the pattern of steady state levels of receptor mRNA, suggesting that transcription of the gene can be directly correlated with the appearance of the receptor protein.
NERVE-TARGET INTERACTIONS IN THE DEVELOPING SYMPATHETIC NERVOUS SYSTEM: DEVELOPMENT OF AN m3 MUSCARINIC CHOLINERGIC RECEPTOR. M.P. Grant and E. C. Landis* Dept. of Pharm. and Center for Neurosciences. Case Western Reserve University, Cleveland OH 44106.

Relatively little is known about the developmental mechanisms which regulate receptor expression. Rat sweat glands provide a model to study the relationship between transmitter phenotype and receptor expression because the innervation changes from adrenergic to cholinergic.

To examine the relationship between gland innervation and receptor expression, we characterized the adult receptor, quantitated the development of muscarinic receptors, and determined the concentration of receptors in two experimental paradigms. In adult glands, the receptor displays high affinity for 4-DAMP, intermediate affinity for pirenzepine, and low affinity for AF DX-116. Preliminary in situ hybridization studies suggest that glands express the m3 molecular subtype (Bonner, T. I. TINS 12: 149). During development the forming glands first express muscarinic binding sites on P4. On P14, shortly after cholinergic properties appear in the innervation, but when only a minority of glands respond to nerve stimulation, the concentration of binding sites approaches adult levels. Glanules that develop in the absence of innervation or glands from adult animals that are acutely desensitized show a reduced concentration of receptors (66% and 80% respectively), of unchanged Kd and are unresponsive to muscarinic agonists. Our results suggest that a significant proportion of muscarinic binding sites are expressed in the absence of functional cholinergic innervation, but that this innervation is important in the development of cholinergic responsiveness in rat sweat glands.


Proteases and their inhibitors are expressed at critical stages in the development of individual neurons and in the overall pattern of development. Inhibition of proteases released from neuronal growth cones decreases cell migration and sprouting, and glial cells can release protease inhibitors. Proteases also have global roles in pattern formation, such as the makle and easter mutanties that affect the dorsal-ventral axis in Drosogphila melanogaster.

The brain of the sphinx moth Manduca sexta undergoes extensive remodeling during metamorphosis, a period when certain larval neurons and networks degenerate and new adult structures form. We used a simple assay to show that stage-6 pupal brain contains protease inhibitor activity for both trypsin and chymotrypsin. Northern analysis at low stringency using a serine protease inhibitor cDNA shows that stage-18 brain and stage-6 antennae contain a 1.6 kb transcript that hybridizes to the 1.4 kb series cDNA.

The protease inhibitors are currently being purified. The cloning of this cDNA and study of its localization will help to determine how proteases and their inhibitors affect the restructuring of the Manduca nervous system during metamorphic adult development. [Supported by grants from the NIH, NSF, and American Cancer Society].


A monoclonal antibody (Mab) 12B3 was produced against a cell suspension from the embryonic rat forebrain. This antibody stained the embryonic and neonatal nervous tissues, but not the adult tissues. In the cerebral cortex of 14 day embryos, the immunoreactivity was detected in the horizontally oriented cells (the first neurons to mature in cortex) in the marginal zone, whereas the glial cells in the ventricular zone were almost negative. In 18 day embryos, the neuronal processes or axons in the intermediate zone and the marginal zone were strongly stained, the glial cells in the ventricular zone being only weakly stained. In the cortical plate and the subventricular zone, the immunoreactivity was gradually reduced in these neural substrates. No reactivity was found in the adult cortex. These results suggest that the spicules detected with Mab 12B3 are transiently expressed in developing nervous tissues.

EXPRESSION OF THE D2-DOPAMINE RECEPTOR IN THE DEVELOPING RAT BRAIN. M.M. Durand*, D.C. Chugani* and M.F. Phelps (SPON: J.C. Mazzioita) Division of Nuclear Medicine and Biophysics, Department of Radiological Sciences, UCLA, School of Medicine, Los Angeles, CA 90024, USA.

As a first step in our effort to produce a cell line containing the D2-dopamine receptor, we have investigated the developmental pattern of the mRNA encoding for the D2-receptor using a cDNA probe (Bunzov J.R. et al., Nature, 336:783, 1985) and the cellular localization of this receptor. During embryogenesis, mRNA was detectable in Northern blot at a low level as early as e12, followed by a subsequent increase in the last stages of embryogenesis. The same developmental pattern can be observed in striatum and mesencephalon. In neuronal cultures from 14-day-old embryonic striatum, the level of mRNA increased significantly during neuronal sprouting (1 to 5 days in culture). The higher level of mRNA was detected at the beginning of synaptogenesis (5 days in culture) and the number of cells mapped by in situ hybridization with specific labelled cDNA reached 40%. Then, during neuronal maturation (5 to 11 days in culture), the level of mRNA plateaued. Using an antibody against the spiperone analog, carboxy-oxime-spiperone, the D2-receptor appeared at 7 days in culture in small segments of neurites. The colocalization of the D2-receptor with specific neuronal markers to identify the cell types containing this receptor in striatum and mesencephalon will be presented.


We studied uptake and retrograde transport of intramuscularly-injected horseradish peroxidase (HRP) by axotomized rat medial gastrocnemius (MG) motoneurons, that were allowed to regenerate but prevented from reinnervating the muscle. The MG nerve was cut and the proximal end sutured onto the normal-innervated lateral gastrocnemius (LG) muscle. To prevent reinnervation, the denervated MG muscle was removed. At various days post-operative (DPO, 7-200), HRP was injected into the LG muscle after the LG-soles nerve was ligated and cut. Cell counts and morphometric measurements were obtained from labeled MG neurons. By 30 DPO and for later DPOs, the somes of MG motoneurons lacking a target were significantly smaller than normal MG motoneurons. The number of labeled MG motoneurons was similar on the control and experimental sides for these same DPOs. At 7 DPO, the reduction of soma size on the experimental side was less evident and the number of labeled neurons on the experimental side was consistently lower than on the control side. The results indicate that motoneurons deprived of functional contact with muscle for prolonged periods atrophy, but do not die.

Supported by NIH grants NS24000 and NS24707.
CO-CULTURES OF TEMPERATURE SENSITIVE PC 12 CELLS AND RAT STRIATAL NEURONS: LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. T. Mihalik, D. Rauch, L.E. Eiden and T.E. Gingerich. Department of Anatomy and Cell Biology, USC School of Medicine, Denver, CO 80262 and Unit on Cellular and Molecular Neurobiology NIMH, Bethesda MD 20892.

PC12 cells undergo neurite outgrowth after transfection with a replication defective recombinant retrovirus containing a temperature sensitive v-src gene. The resultant cells (PCB8-T1) proliferate at the non-permissive temperature of 40°C, but stop dividing and differentiate at 37°C (Rauch and Eiden, Abst. 88). The purpose of the present study was to characterize contacts between differentiated PCB8 cells and cultured rat striatal neurons.

Stria were dissected from E14 fetal brains and were mechanically disrupted and plated in 10 cm dishes. After 7-14 days, 1 x 10⁴ undifferentiated PCB8 cells were added to the dishes and were grown at 37°C for an additional 7-10 days. Cells were fixed and prepared for electron microscopy and immunocytochemistry.

PCB8-T1 cells extended extensive networks of neurites within 7 days of coculture. The majority of PCB8-T1 processes were confined to areas of plates which were very heavily occupied by undifferentiated PC12 cells and their ability to respond to NGF. We have overexpressed ODC in PC12 cells by two independent strategies. Cells have been grown in increasing concentrations of diethylstilbestrol to induce the synthesis of ODC, and cloned using ODC isolated from untransformed PC12 cells. Both cloned cells, cells are dramatically altered. They are extremely flattened with very prominent nuclei and nucleoli. ODC transfecants fail to project neurites in response to NGF. Transfection of the same DNA and mRNA into PC12 cells contained relative to the promotor yields cells that do project neurites when treated with NGF. We have also stably introduced the c-myc gene into PC12 cells. Clone ODC transfecants display similar traits as the ODC-expressing cells, i.e., flat morphology and failure to project neurites in response to NGF.

This study identifies two genes whose excess activity alters cell morphology and responsiveness to NGF in a similar manner. It is possible that each influences a common biochemical pathway. Further investigation of these effects may lead to a better understanding of the mechanisms of NGF action and neuronal differentiation. (Supported by the Muscular Dystrophy Association and NIH Grant #RO1-NS24367)

MORPHOLOGICAL ALTERATION AND INHIBITION OF NGF INDUCED NEURITE OUTGROWTH IN PC12 CELLS OVEREXPRESSING C-MYB OR ORNITHINE DECARBOXYLASE. S.C. Feinstein, L.G. Marshall* and G. Yezerov* Neuroscience Research Institute and Dept of Biological Sciences, University of California, Santa Barbara, CA 93106.

We have examined the effects of two gene activities, ornithine decarboxylase (ODC) and c-myc, on PC12 cells and their ability to respond to NGF. We have overexpressed ODC in PC12 cells by two independent strategies. Cells have been grown in increasing concentrations of diethylstilbestrol to induce the synthesis of ODC, and cloned using ODC isolated from untransformed PC12 cells. Both cloned cells, cells are dramatically altered. They are extremely flattened with very prominent nuclei and nucleoli. ODC transfecants fail to project neurites in response to NGF. Transfection of the same DNA and mRNA into PC12 cells contained relative to the promotor yields cells that do project neurites when treated with NGF. We have also stably introduced the c-myc gene into PC12 cells. Clone ODC transfecants display similar traits as the ODC-expressing cells, i.e., flat morphology and failure to project neurites in response to NGF.

This study identifies two genes whose excess activity alters cell morphology and responsiveness to NGF in a similar manner. It is possible that each influences a common biochemical pathway. Further investigation of these effects may lead to a better understanding of the mechanisms of NGF action and neuronal differentiation. (Supported by the Muscular Dystrophy Association and NIH Grant #RO1-NS24367)

MODULATION OF GATE-CLAMINES IN RETINOBLASTOMA CELLS INDUCED TO DIFFERENTIATE BY RETINAL PIGMENTED EPITHELIAL CELL CONDITIONED MEDIUM. L. Tombran-Tink, L.Y. Johnson, L. Adams* and L. Kliman. Department of Anatomy & Cell Biology, USC School of Medicine, Los Angeles, CA 90033.

Previously we described the induction of neuronal differentiation of human Y79 retinoblastoma cells by medium conditioned by human retinal pigment epithelial cells (RPE-CM). We have examined modulation of catecholamine-containing cells in undifferentiated, differentiated Y79 retinoblastoma cells by HPLC. These analyses reveal decreased levels of dopaminergic as well as noradrenergic catecholamines in differentiated Y79 cells. Dopamine is present at 25 picomoles/mg in non-differentiated Y79 cells while epinephrine is undetectable. In contrast, differentiated Y79 cells show undetectable levels of dopamine but increased epinephrine levels to 297.8 pm/mg and trace levels of norepinephrine. We have further observed that epinephrine levels are elevated by 4-8 days exposure to RPE-CM, prior to attachment and differentiation. Such "stimulated" cells contain 140.6 pm/mg epinephrine and low levels of putative norepinephrine while dopamine content is already decreased to undetectable levels. Indeed, some such dopamine B-hydroxylase and norepinephrine N-methyltransferase may be activated during the differentiation process. (Supported by NIH EY07671.)

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: TISSUE CULTURE MODELS FRIDAY AM

Tissue plasminogen activator binds to neuronal thy-1. J. H. Wang* and B. N. Pitman* (SPON: G. B. Kocile) Mahoney Institute for Neurological Sciences and Dept. of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Plasminogen activators (PA) are a class of serine proteases which convert the zymogen plasminogen into the fibrinolytic proteinase plasmin. PAs have been implicated in many processes, including development, extravasation, and tumor migration, among others. Several preliminary data suggested that the binding site might be the neuronal glycoprotein Thy-1. Binding of 125I-PA to PC12 cell surfaces indicated a single PA binding site with a Kd of 1 × 10⁻⁸ M. This and other preliminary data suggested that the binding site is on the neuronal surface. The 125I-PA binding properties of purified rat brain Thy-1 were then compared to those of PC12 cells, which specifically bind 125I-PA to Thy-1. 125I-PA bound to Thy-1 with a k1 = 28 ± 10⁻⁷ min⁻¹ and a K1 = 1.4 × 10⁻⁷ M⁻¹. The k - 1 values were 7.1 × 10⁻³ min⁻¹ for Thy-1 and 6.5 × 10⁻⁷ min⁻¹ for PC12 cells. Kinetically determined K1 values were 25 pm for Thy-1 and 1 × 10⁻³ for PC12 cells. The proteolytic site of PC12 does not appear to be involved in binding to Thy-1. The involvement of other domains of PC12 is being investigated, as is the functional significance of this interaction. Supported by the McKnight Foundation and NIH NS22663.

CORTICOSTEROID-INDUCED NEURITE OUTGROWTH IN PC12 CELLS. A. J. Tombran-Tink* and L.Y. Johnson (SPON: M. Colvin).* Department of Anatomy & Cell Biology, USC School of Medicine, Los Angeles, CA 90033.

Y79 human retinoblastoma cells can be induced to differentiate along neuronal pathways by medium conditioned by fetal retinal pigment epithelial cells (RPE-CM) (Tombran-Tink and Johnson, Invest Ophthalmol. Vis. Sci. 23:141, 1982). We have examined the expression of several neuronal markers during RPE-CM-induced neurogenesis of Y79 cells. The probes employed were conefromospecific monoclonal antibodies 1 and 2 (OVA-1, OVA-2) and polyclonal antiserum to cone photoreceptor-specific proteins. In the presence of neuron-specific enolase (NSE) expression in the cells studied, the expression of neuronal markers could be correlated with a known neuronal marker. Immunoreactivity to OVA antibodies was detected in undifferentiated Y79 cells and not in differentiated cells, in contrast to the findings of others, were extended here to include excitable membrane properties. Thus, differentiated Y79 cells express a spectrum of features characteristic of neurons. Our findings indicate that neuronal differentiation of retinoblastoma cells involves the expression of several molecular species characteristic of cone photoreceptor cells in the retina. (Supported by NIH EY02662.)

CORE PHOTOSENSOR-SPECIFIC MOLECULES EXPRESSED BY NEURONALLY DIFFERENTIATING Y79 HUMAN RETINOBLASTOMA CELLS. G. Massry*, L. Tombran-Tink* and L.Y. Johnson (SPON: M. Colvin).* Department of Anatomy & Cell Biology, USC School of Medicine, Los Angeles, CA 90033.

The P19 embryonal carcinoma cell line was used as a model for examining the development of ionic currents in the differentiation of pluripotent cells toward a neuronal phenotype. Retinoic acid (RA; 0.1 µM) was used as the differentiating agent (Jones-Villeneuve et al., J. Cell. Biol. 94, 1982). RA induced cell differentiation and a 100-fold increase in the expression of neuronal specific antibodies in these cells, stilts that expression of ionic currents could be correlated with a known neuronal marker. Immunoreactivity to OVA antibodies was detected in undifferentiated Y79 cells and not in differentiated cells, in contrast to the findings of others, were extended here to include excitable membrane properties. Thus, differentiated Y79 cells express a spectrum of features characteristic of neurons. Our findings indicate that neuronal differentiation of retinoblastoma cells involves the expression of several molecular species characteristic of cone photoreceptor cells in the retina. (Supported by NIH EY02662.)


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524.7 NEURONAL ULTRASTRUCTURAL CHARACTERISTICS OF A CHOLINERGIC CELL LINE. D.N. Hammond, H.J. Lee, and B.H. Water. Departments of Pediatrics and Neurology and the Committee on Neurobiology, The University of Chicago, Chicago, IL 60637.

A hybrid cell line expressing differentiated characteristics typical of cholinergic neurons has been derived from the murine neuroblastoma cell line LA-N-1. In addition to cholinoceptive and cholineacetylase activity and the expression of neurotransmitter, SN17 cells display neuronal morphological characteristics at the light microscopic level. We have undertaken a detailed study to determine the ultrastructural traits expressed by the line. Cells are grown in Delforge's modification of Eagle's medium containing 10% fetal calf serum. They are fixed with 0.5% paraformaldehyde and 2% glutaraldehyde, postfixed with osmium tetroxide, stained with uranyl acetate and lead citrate, and examined in a Philips 201 electron microscope. The soma contained numerous mitochondria and prominent rough endoplasmic reticulum. Membrane specializations, with maintenance of the intercellular cleft but no adjacent vesicles or external appendages. The neurites displayed a prominent microfilaments containing, in addition to intermediate filaments and microtubules, clear and dense core vesicles, and vesicles. SN17 cells thus express, under culture conditions, and in the absence of any differentiating agents, a number of ultrastructural characteristics typical of neurons. (Supported by NIH grants NS 01324, NS 25787, and T32HD070009, and grants from the Department of Public Health and the Alzheimer Disease and Related Disorders Association.)


The neuroblastoma cell line LA-N-1 has previously been characterized as adrenergic based on tyrosine hydroxylase (TH) activity and immunocytochemical staining for TH, dopamine beta hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT). Although enzyme assays showed no activity for DBH or PNMT, we demonstrated that staining for TH and DBH increased after induced cellular differentiation. To explore this discrepancy between methods, we used a highly sensitive HPLC assay to test for the storage of catecholamine products. We found that these cells do not contain dopamine, but do contain norepinephrine (NE), epinephrine (E) and a significant amount of serotonin (5HT). The amount of neurotransmitter product stored is dependent upon the differentiation state of the cells. Mitotic cells contain 17.8 pmol NE/mg protein, 34.4 pmol E/mg protein and 52.4 pmol 5HT/mg protein. After differentiation for 4 days with 10^{-5} M retinoic acid, the cells contain 68.1 pmol NE/mg protein, 261.3 pmol E/mg protein and 10.79 pmol 5HT/mg protein. Since we have previously shown that LA-N-1 cells are sensitive to 1-methyl-4-phényl-1,2,3,6-tetrahydropyridine (MPTP) in a dose dependent manner and many clinical lines contain monoamine oxidase (MAO), we explored the possibility that these cells possess MAO activity. Initial studies using a fluorosecent method utilizing kynurenine as substrate show MAO activity in both mitotic and chemically differentiated cells. Preliminary results indicate that these cells might contain both MAO type A and B, although this remains to be fully substantiated. (Supported by NIH NS 25787)

524.9 IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL CHARACTERIZATION OF ORGANOTYPIC SLICE CULTURES OF CEREBRAL CORTEX GROWN IN DEFINED MEDIUM. C.M. Annis, R.T. Robertson and J. Yu. Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, University of California, Irvine, CA 92717.

We are studying the development of cerebral cortex and certain cortical afferents, using the organotypic tissue slice culture method of Gahwiler (TINS, 1988). We report here the characterization of cortical neurons that survive in culture, with regard to immunocytochemical and histochemical staining properties. Slices of parieto-temporal cortex were obtained from rat pups aged 3-5 days. Slices were cultured on collagen coated coverslips in defined medium (Annis et al. 1989) for 1-4 weeks. Following fixation, cultures were processed for acetylcholinesterase (AChE), cytochrome oxidase (CO), choline acetyl transferase (ChAT), gamma-aminobutyric acid (GABA), glutamate, gial fibrillary acidic protein (GFAP) and a number of other ultrastructural and histochemical agents. Cultures of cortex grow well in defined medium and maintain normal gross cortical appearance. Histochemical and immunocytochemical studies demonstrate that a variety of types of neurons survive in culture. Neurons stain for AChE or ChAT, but AChE-positive neurons are found more frequently in culture than in vivo. Multinuronal neurons stained for GABA, whereas neurons with pyramidal morphologies stained for glutamate. These results indicate that cortical slice cultures maintain many of the same peptides and neurotransmitters that are found in vivo, and provide a promising model for the study of cortical development. Supported by NSF grant 87-08515 and NIH grant NS 25674.

525.1 ORGANIZATION OF THE PERIPHERAL PROJECTIONS OF THE TRIGEMINAL GANGLION IN FETAL RATS. G.J. Macdonald, N.L. Chiaia. Departments of Pediatrics and Neurology and the Committee on Neurobiology, The University of Chicago, Chicago, IL 60637.

Retrograde tracing with true blue (Tß) and diamidino yellow (DY) was used to evaluate the topographic organization of the peripheral projections of the trigeminal (V) ganglion in rats on embryonic day 16 (E-16). Timed pregnant rats were killed at this stage of development by cervical dislocation and the brain was rapidly removed. E-16 fetuses were exposed, and small injections of Tß and DY were made into the face. After 8-12 hr, fetuses were harvested and tissue was prepared for fluorescence microscopy. At E-16, peripheral V ganglion projections were quite adult-like. Cells that projected to the vibrissa pad were restricted to the ophthalmic-maxillary part of the ganglion with those innervating dorsal (A- and B-) and ventral (D- and E-) rows located medially and laterally and projections to the vibrissa pad were restricted to the lateral and ventral part of the ganglion. None of the combinations of injections that we carried out produced large numbers of double labelled cells. Small numbers (<15 per ganglion) of double labelled cells were seen after injection of Tß into the vibrissa pad and DY into the vibrissa pad. These results indicate that the peripheral projections of the V ganglion are quite adult-like from the earliest stage at which we can retrogradely trace them with these agents. Supported by DE 07734, NBS 85 17537, and funds from the State of Ohio Research Challenge.

SOMATOSENSORY SYSTEM II

525.2 DEVELOPMENT OF INPUT PATTERNS FROM THE DIGITS TO THE SPINAL CORD AND CUNEATE NUCLEUS IN MONKEYS. Florence, S.L., and J.B. Kaas. Dep. Psychol. Vanderbilt Univ., Nashville, TN 37240. Injections of transganglionically transported HRP conjugates into the digit tips of a 5 day and 1 month old macaque monkey (Macaca fascicularis) demonstrated exuberant terminations of peripheral nerve afferents in the dorsal horn of the spinal cord but adult-like restrictions of terminations in the cuneate nucleus of the brainstem. In the spinal cord of the neonate, terminations extend rostrocaudally across almost two thirds a cortical segment in the superficial dorsal horn and span 2 segments deeper in the dorsal horn. The deeper label is less dense and discontinuous, forming a sequence of small (100mu) patches of label separated by unlabeled gaps of comparable size. A similar pattern was found in the dorsal horn of the 1 month old monkey. In adults (Florence, et al., 1989), terminations are found only in the superficial dorsal horn and are restricted to less than half a segment. In the pars rotunda of the cuneate nucleus, digit injections result in discrete foci of label restricted within specific cytochrome oxidase dense clusters in both the neonate and adult monkeys. Rostrocaudally and caudally, the label is less dense and more widespread. Thus, the pattern of inputs to the dorsal horn, even one month after birth, is not as discrete as in adults. Yet, by birth, projections to the cuneate nucleus are adult-like. (Supported by NS16446 and NS38082).
525.3 ROLE OF POSTNATAL PRIMARY AFFERENT ACTIVITY IN CENTRAL TRIGEMINAL PATTERN FORMATION. T.A. Henderson*, T.A. Woolsey & M.F. Jacquin (SPON: L.C. Massopust). Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Infraorbital injury at birth reduces primary afferent inputs to ventral trigeminal (PrV) terminal fields, and sensory projections to the thalamus. PrV neurons are densely stained in the face and jaw region of the newborn brainstem, especially in the contralateral area, and PrV terminal fields do not appear to be reduced below normal. In newborns, PrV neurons exhibit increased synaptic density and number of perisomatic spines. In young and adult rats, PrV terminal fields are smaller than in newborns, and perisomatic spines are reduced. These results suggest that primary afferent activity may play a role in the pattern formation of the trigeminal system.

525.4 NEONATAL INFRASPINAL NERVE SECTION: DIFFERENTIAL EFFECTS ON TRIGEMINAL BRAINSTEM CELL NUMBER, SIZE AND DISTRIBUTION IN RATS. T.A. Clugston*, T.A. Henderson & M.F. Jacquin (SPON: L.C. Massopust). Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Neonatal infraspinatal nerve section produces a 21% reduction in HRP-labeled projection cells in the trigeminal brainstem complex. Differential morphometric methods were used to replicate this finding and to estimate differences in local circuit cell number and distribution. Nissl-stained nuclei in 10 um parasagittal sections were reduced in number by 31, 35, and 46% in principalis, interpolaris, and oralis subnuclei, respectively. Such differential cell counts in spinal vs. principalis suggest that infraspinatal nerve section may produce changes in the distribution of neurons in higher order trigeminal relays. This finding is consistent with the hypothesis that peripheral inputs play a role in the development of the trigeminal system.

525.5 NEONATAL INFRASPINAL NERVE SECTION: EFFECTS ON CAUDALIS CELL STRUCTURE-FUNCTION RELATIONSHIPS. M. Barcia* & M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis University School of Medicine, MO 63104.

Infraorbital nerve injury at birth produces changes in topography, inputs, projection status, cell number, and responses of interneurons in the trigeminal system. To determine whether receptive field changes reflect altered somadendritic development, intracellular recording, receptive field mapping, and HRP injection techniques were applied to 43 adult interpolaris and 54 normal cells. Thus, deafferented cells tended to have more proximal dendrites and radially oriented, oblique dendritic trees with normal transverse areas. Support: NIH DE07734, DE07662.

525.6 NEONATAL INFRASPINAL NERVE SECTION: EFFECTS ON INTERPOLARIS CELL STRUCTURE-FUNCTION RELATIONSHIPS. M.F. Jacquin & T.A. Henderson. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Deafferentation at birth produces changes in topography, inputs, projection status, cell number, and responses of interneurons in the trigeminal system. To determine whether receptive field changes reflect altered somadendritic development, intracellular recording, receptive field mapping, and HRP injection techniques were applied to 43 adult interpolaris and 54 normal cells. Thus, deafferented cells tended to have more proximal dendrites and radially oriented, oblique dendritic trees with normal transverse areas. Support: NIH DE07734, DE07662.

525.7 NEONATAL INFRASPINAL NERVE SECTION: ULTRASTRUCTURAL COMPARISONS OF NORMAL AND DEAFFERENTED NUCLEUS PRINCIPALIS IN THE RAT. J. Golden, D.S. Zahn & M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis University School of Medicine, MO 63104.

Infraorbital nerve injury at birth reduces primary afferent inputs to ventral trigeminal (PrV) terminal fields, and sensory projections to the thalamus. PrV neurons are densely stained in the face and jaw region of the newborn brainstem, especially in the contralateral area, and PrV terminal fields do not appear to be reduced below normal. In newborns, PrV neurons exhibit increased synaptic density and number of perisomatic spines. In young and adult rats, PrV terminal fields are smaller than in newborns, and perisomatic spines are reduced. These results suggest that primary afferent activity may play a role in the pattern formation of the trigeminal system.


Principalis cells exhibit orderly structure-function correlations and topography, require peripheral inputs for normal development, and establish patterns in higher-order trigeminal structures. As a first step in assessing mechanisms underlying these changes, we have studied in adulthood and at 3 postnatal days (PN4) when naturally occurring cell death and primary afferent segmental retraction take place in side afferent (birth-PrV) and efferent (PrV) tracts. Brainstems from 4 animals of each age were processed using standard Golgi-Cox methods. 40-50 cells of each age were analyzed. Principalis neurons of all ages were large, had numerous spiny dendrites with extensive local axon collaterals, and they were closely packed. The principalis cells observed in animals at 3 PN4 had undergone morphological and immunocytochemical changes that were consistent with the hypothesis that peripheral inputs play a role in the development of the trigeminal system.

525.9 SOCIOITY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

FRIDAY AM

1332 SOMATOSENSORY SYSTEM II

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NEONATAL INFRAORBITAL NERVE SECTION: EFFECTS ON TRIGEMINAL SECOND-ORDER COLLATERAL PATTERN ASSOCIATED WITH MULTIPLE RETROGRADE TRACERS.

A. P. Young, T. A. Henderson, N. L. Usowicz, M. A. Gross, and D. S. Knight.* Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

The purpose of this study was to determine the neurogenic period for projection neurons of the ascending tracts. In order to label projecting neurons, tritiated thymidine was administered to fetal rats on embryonic (E) days E12 through E15. Ascending tract neurons of the lumbar cord were later (postnatal days 40-50) labeled with Fluoro-Gold applied at the site of a lesion on the spinal cord segment C3. Ascending tract neurons which were undergoing mitosis in the upper lumbar cord were double labeled, i.e., labeled with both tritiated thymidine and Fluoro-Gold. Double labeled neurons were observed from days E12 through E15. The maximum number of double labeled neurons, 41%, was observed on day E13. The number diminishes to 1 to 4% on day E15. Double labeled neurons on day E15 were confined to laminae III, IV, V, and X, and the Nucleus dorsalis and most had ipsilateral projections. Results show that ascending tract neurons continue to proliferate throughout most of the neurogenic period and that most of the neurons undergoing mitosis on the final day of proliferation project ipsilaterally.

$52.10$

THE DISTRIBUTION OF SPINAL AFFERENTS IN THE THALAMUS AND HYPOTHALAMUS OF NEONATAL RATS.

S. Henderson, Department of Anatomy and Neurobiology, University of Florida, Gainesville, FL 32610.

The normal distribution of spinal afferents to the thalamus was examined in 0 and 5 day old rats using the anterograde transport of horseradish peroxidase conjugated wheat germ agglutinin dissolved in radiocontrast medium injected into the cervical spinal cord 48-72 hours earlier. Reaction product from anterograde transport was present, in animals killed on postnatal day 3, in the posterior group; parafascicular, central medial and central lateral nuclei; submedius and the zona incerta. A wide extent of the ventral posterior lateral n. (VPL) was labeled with anterograde reaction product. Anterograde reaction product was also visible in the hypothalamus in the paraventricular n., the lateral and posterior hypothalamic areas. A few axons were visible passing through the periventricular area. Labeled somata were present in the paraventricular n., the lateral hypothalamus and in zona incerta. A periventricular pattern of label was present on PND 5, though there appeared to be some refinement of the labeling pattern.

DEVELOPMENTAL DISORDERS: HUMAN DISEASES

$52.11$

NEUROGENESIS OF SPINOCEBERELLAR NEURONS IN THE LUMBAR SPINAL CORD OF THE RAT.

J.A. Real, F.N. Mandl*, and D.S. Knight*. Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

Studies in our laboratory show that ascending tract (relay) neurons of the rat spinal cord proliferate from embryonic (E) day E12 through E15. Relay neurons which proliferate on day E15 represent only a small percentage of the total population of ascending tract neurons and are observed in spinal cord laminae which give rise to major cerebellar projections. The objective of the present study, then, was to determine if the late dividing relay neurons on day E15 also gave rise to major cerebellar neurons. In order to label these cells during their proliferative period, tritiated thymidine was administered. Serial sections on day E15 were labeled, i.e., labeled with both tritiated thymidine and Fluoro-Gold. Double labeled neurons were later labeled in each animal via the retrograde transport of Fluoro-Gold, which was injected unilaterally into the cerebellar hemisphere. Cell counts showed that 25% of the spinocerebellar neurons in the upper lumbar spinal cord were labeled with tritiated thymidine on E15. This is in contrast to 1 to 4% of the total population of ascending tract neurons labeled with tritiated thymidine at this time. In conclusion, spinocerebellar neurons represent a large proportion, if not all, of the relay neurons dividing on the final day of spinal neurogenesis for ascending tract neurons.

Perinatal and infantile dopamine-beta-hydroxylase (DBH) deficiency is a newly recognized disorder in which patients fail to express sufficient quantities of the functional enzyme. Consequently, they have little or no norepinephrine and epinephrine in the central or peripheral nervous system. In contrast, dopamine, the substrate for DBH, is present in five to tenfold excess. Metabolites of the catecholamines show the same trends as the primary amines. The physical characteristics of the patients include orthostatic hypotension, cardiovascular insufficiency, ptosis of the eyelids, hyperextensible joints, and retrograde ejaculation. Patients appear normal in development and in intellectual function. Peripherally noradrenergic neurons are responsive to appropriate stimuli but release dopamine rather than norepinephrine.

To determine familial DBH deficiency is by gross alterations at the DBH locus, we analyzed lymphocytic DNA from DBH deficiency patients and their families, following digestion with Bam HI, Eco RI, and Tag I and subsequent hybridization of a radiolabeled human DBH cDNA. We observed no alterations in restriction patterns in any family members. These findings indicate that DBH deficiency in these families is not due to gross alterations such as insertions or deletions of the DBH alleles. Following digestion with Tag I, we detected an apparent restriction fragment length polymorphism. Further analysis of this polymorphism in additional DBH deficient families will be required to determine if alteration of the DBH gene is present in DBH deficiency.


Hydrocephalus was induced in 4-10 day old kittens by intracisternal injection of kaolin. Hydrocephalic animals were killed at 10-12 days post-kaolin. Ventriculograms was confirmed by imaging procedures before the animals were killed. Tissues from cortex (areas 4, 17, 22), basal forebrain and hippocampus were processed for electron microscopy and immunohistochemistry. Deep layers of cortex were severely atrophied, with marked alterations in neuronal and dendritic morphology including reduction in synaptic contacts. Destruction of neuropil was less in superficial layers, but synaptic numbers were decreased. Dendritic morphology was correlated with observations made on age-matched Golgi impregnated cortex. The decrease in spineous profiles parallels similar observations in Golgi material. Substance P, Leucine enkephalin, and ChAT distributions were studied using routine immunohistochemical procedures. Cells in deeper layers gave origin to fiber systems within more superficial layers which survived hydrocephalus but the fibers were compressed in the remaining neuropil.

ABSENCE OF BRAINSTEM ABNORMALITY IN STUDY OF AUTISTIC PATIENTS USING MAGNETIC RESONANCE IMAGING. M. Hose*, E. Courchesne*, and G. Press* (SPON: V. Kool). Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92039.

In vivo studies using magnetic resonance imaging (MRI) indicate that autism is often associated with developmental hypoplasia of the neocerebellar vermal lobules VI and VII. Recent electrophysiological and neuropathological studies have produced conflicting evidence for brainstem involvement in autism. In our study, we tried to replicate previous findings (Gaffney et al, Biol Psychiatry, 1988) that the pons was significantly smaller in autistics compared to controls. MRI scans were obtained from 41 autistics (mean age = 17.9 ±1.4, range = 2 to 29 yrs) and 36 normal controls (mean age= 20.6 ± 1.6, range = 3 to 29 yrs). Scans were evaluated by two experienced raters who were blind to the status of the subjects. Five autistic subjects had polymericgria, one had schwannoma, and two normal. None of the control subjects had abnormalities of this type on MRI. Polymericgria, schwannoma, and monoplastic agenesis are part of a related group of cortical malformations resulting from anomalous development of the cerebral cortex prior to six months gestation. In addition, areas of the cerebellar cortex was present in four autistic subjects and one control. Structural abnormalities of the cerebral cortex may be common in autism and suggest the possibility that neuronal migration defects may be an important mechanism in the pathophysiology of autism.


We performed an immunocytochemical study on postmortem brain obtained from a 21 year old retarded woman with clinical features typical of autism who died of pneumonia. Several small cortical blocks and a sample of the amygdala of the left hemisphere were selected for analysis. The remainder of the hemisphere was processed for celloidin embedding and whole brain sections while the right hemisphere was frozen for future neurochemical studies. Glibal fibrillary acidic protein (GFAP) histochemistry showed extensive gliosis throughout all amygdaidal nuclei, C4A and C43 fields of hippocampus, and deep layers of cortical samples from Brodmann areas 39,40,41,42,49 (including Wernicke's area). Samples of superior frontal, superior parietal, frontal, and temporal cerebral cortex also showed subpial gliosis and GFAP positive astrocytes scattered in the white matter. Neuronal/glial and postnatal reactive immunoreactive pyramidal neurons and parvalbumin immunoreactive local circuit neurons did not appear abnormal in the gliotic cortical regions. The localization of abnormalities in the amygdala and hippocampus is consistent with the previous postmortem study and is consistent with the behavioral abnormalities typical of autism. The attenuation of language related cortical areas suggests an underspecification of the clinical language disturbance as well. The distinctive distribution of gliosis in this case, therefore, suggests one possible pathologic correlate of autism.
MANAGING PERSISTENT REPEETITIVE BEHAVIOR: DEVELOPMENTALLY / NEUROLOGICALLY DISABLED, INC. 
14155 W. 103rd Place, M.C. Wetzell, M.J. Taylor, and M.J. Lachowski* 
Psych Dept., Univ. Ariz., Tucson, AZ 85721 and Desert Sun* 
Desert Sun, Tucson, AZ 85751.

Many developmentally disabled persons—a majority at the Desert Survivors day program—have recurrent, stereotyped behaviors. Central pattern generator neurons may be pathologically active, but any such mechanism is still highly susceptible to stimulus control and new learning. Daily progress notes and continuous behavior records formed a computer-organized data base. Training interventions based on pattern and rhythm in a given context promoted more harmonious social work skills. (1) Rapid, persistent rocking or gestures repeating at 1 or 2 per sec dropped out during organized activity [shovelling; stacking pots; eating]. (2) Staff built rhythmic manual skills [e.g. filling pots with earth], having an orderly onset and completion [see provided when pot was full], from dysfunctional acts [lossing dirt]. (3) Occasional but strong hitting, rocking, or self-abuse also responded when the entire action was guided smoothly to a safer place away from the work crew where its strength could be reduced by stages. These rhythm- and context-appropriate interventions succeeded where traditional behavior modification of isolated responses had failed.

COMPREHENSION AND EXPRESSION OF AFFECT IN LANGUAGE IMPAIRED CHILDREN. D.A. Trauner, A. Ballontyne* 
Cf. Chabot and Z. Talmam, *Dept. of Neuroscience & Psychiatry, Univ. of CA, San Diego, CA 92093.

In addition to their delays in acquiring language, developmental language impaired (LI) children also may have difficulties with affective expression and communication. We studied 7 LI children and 7 age, sex, and IQ-matched controls to determine whether there were any differences in their comprehension and expression of affective intonation through spoken language or facial expression. Each child was tested on the ability to 1) identify emotions in spoken phrases (e.g. sadness and happiness); 2) imitate verbal phrases using the same emotional intonations; 3) complete stories using the appropriate spontaneous emotional intonations; 4) select which emotions are conveyed in facial photographs; 5) imitate those facial expressions; and 6) spontaneously produce the appropriate facial expressions in response to stories. Compared to controls, LI children performed significantly more poorly on tasks involving verbal comprehension and expression (R,2.3.1). LI's performed better than controls on production of emotional facial expressions (R5.6.1). The problem with affective communication in LI appears to be modality-specific. Their superior performance on visual tasks may reflect overcompensation for their auditory deficits through the use of an intact visual communication system.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY VI

LATE POSTNATAL EMERGENCE AND PROTRACTED DEVELOPMENT OF ACETYLCHOLINESTERASE IN MEYNERT SOLITARY CELLS OF THE HUMAN VISUAL CORTEX. L. Kostovic* 
Dept. of Anatomy, All India Inst. Medical Science, New Delhi, India 110029.

The maturation of Meynert cells in the human striate cortex was studied by use of an AChE histochemical method on postmortem brain tissue obtained from individuals ranging in age from birth to 13 years. During all stages the striate area was sharply delineated by the presence of an AChE reactive band in layer IV. Although well developed Meynert cells with large pyramidal-shaped somata situated at the interface to their connections with medial temporal association cortex. Supported by NIH grants NS 14841 and NS 2280.


An antibody to the GABA receptor (Incastor Co.) was used in conjunction with a monoclonal antibody (MabE9; gift of J. Talman, Yale University) to study the developmental expression of GABA receptor subunits. Immunoreactivity to BZD receptors was first seen at 16 months of age. Similarly, by 2 years more than 90% of the perikarya of Meynert neurons are weakly AChE positive. Between 2 and 7 years there is an increase in staining as moderate AChE reactivity extends to proximal dendrites of these cells. By 10 years all Meynert cells are heavily stained and display thick horizontal dendrites. Thus, this solitary class of giant pyramidal neurons expresses a receptor complex related to their connections with medial temporal association cortex. Supported by Yugoslav-U.S. Joint Board.


*Anatomy Institute, Univ. Kiel, Kiel, West Germany.

We localized immunoreactivity for two calcium-binding proteins, calbindin (CaI) and parvalbumin (PV) in neurons of Macaca nemestrina visual cortex. In adult cortex, 90% of GABA neurons contained either CaI or PV, but very few cells contained both. Of the GABA+ neurons, 15% contained CaI and 75% contained PV. Most heavily-labeled CaI+ neurons occurred in layers 2/3 with a few in layers 5/6. PV+ cells are seen in all layers, but the number peaks in layer 4. Diameters of CaI+ cells always were significantly smaller than PV+. PV+ axons run in white matter under striate but not peristriate cortex which can be traced into layer 4. This differential distribution suggests functional differences within the GABA population.

During development, GABA+ neurons are found at fetal (F) 60d, but PV+ cells are first seen at F152d in the deep layers. By postnatal (P) 1-3 days, PV+ neurons are visible and, by P10, PV+ axons are found in all layers except upper 2 and 1 with PV+ axons in white matter. The adult pattern is not seen until P6 months. In contrast, CaI+ neurons are numerous in CaI and 152d and are seen there until after birth. Most cortical layers contain both stellate and pyramidal CaI+ neurons by F152d. Subsequent development has a complex laminar specific pattern, with the adult pattern localization only in stellate neurons not found until P6-12 mo. We conclude that the late appearance of PV may correlate with the onset of visual function while CaI must have a role(s) during development as well. (Supported by EY01208, EY04536 & EY07031).


We have staining sections of Macaca nemestrina visual cortex with a polyclonal antisera rabbit raised against substance P (PoSPas; Incstar Corp). Frozen sections of aldehyde fixed cortex were obtained at fetal days (F) 125 and 162 and postnatal (P) 1 day, 3, 9, 20 wk and adult. A monoclonal SPas (Mehra and Hendrickson, Soc.Neurosci.1987) stained stellate cell bodies, dendrites, axonal processes and synaptic terminals. In contrast, the PoSPas stains larger than the spiny stellate terminals which exhibited morphological characteristics typical of small pyramidal cells, including apical dendrites which extended to lamina 2. Their cell body diameters ranged between 6.5-12.5 µm. PoSPas also labeled large pyramidal axons and terminals which are stained neuronal perikarya in infragranular layers. The PoSPas stained neuronal perikarya in infragranular layers. Subsequent development has a complex laminar specific pattern, with the adult pattern localization only in stellate neurons not found until P6-12 mo. We conclude that the late appearance of PV may correlate with the onset of visual function while CaI must have a role(s) during development as well. (Supported by EY01208, EY04536 & EY07031).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
IMMUNOCYTOCHEMICAL STUDIES OF PROTEIN KINASE C ONTOGENY IN CAT VISUAL CORTEX

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Protein kinase C (PKC) is a major intracellular messenger in brain. It is activated by the inositol lipid hydrolysis and is involved in the phosphorylation of many proteins including myelin basic protein, MAP-2, GAP-43 and various receptors. Many of its important roles in the development of the central nervous system. In the present work, a polyclonal monospecific antibody against PKC was applied to investigate the ontogeny of the kinase in cat visual cortex with immunocytochemistry. PKC immunoreactivity was always found in layer 2,3,5,6 with lower densities in layers 4 and 1. However, the staining of the between layers varied during development. Cells in layers 2/3 showed a greater amount than layer 5/6 at 20-40 days postnatal. Therefore, the ratio of PKC in the visual cortex was in a dynamic pattern. The comparison between the present results and those with [3H]Phorbol ester autoradiography is discussed.

Laminar patterns of acetylcholinesterase (ACHE) activity in primary visual cortex differ in developing and adult rats. In this study, the pattern of ACHE in visual cortex was compared with the distribution of geniculocortical terminals and cytochrome oxidase (CO) activity in developing and mature rats. Infant (P2-P10) and adult rats were injected intracerebrally with 1-10% WGA-HRP. After 3-5 days, animals were perfused and sections processed for ACHE, CO, or HRP histochemistry.

In visual cortex of both developing and adult rats, intracortical injections of WGA-HRP result in anterograde translabeling of geniculocortical terminal fields in layers IV and deep III, and to a lesser degree, layers I and VI. In developing animals (P6-P13) the laminar pattern of endogeneous ACHE reactivity closely resembles that observed in adult rats. In younger animals (P1-P4) the transient ACHE is not present, but elevated CO is found in subplate neurons. In visual cortex of adult rats, ACHE is found primarily in layers V, deep IV, and I, and is more closely associated with geniculocortical terminals in developing, but not adult, rat visual cortex. Elevated CO, first in subplate neurons and later in neurons of layer III-IV, is more closely associated with developing oxidative metabolism of neurons postsynaptic to geniculocortical terminals.

Supported by NSF grant 87-08515 and NIH grant NS 25674.
527.17


Following neonatal cerebral hemispherectomy cats show sparing of the contralateral visual field. Considering the current understanding of the "Sprague Effect," we wished to determine if these animals show a reorganization of the corticotectal projection arising from the primary visual cortex (areas 17 & 18). WGA-HRP (5%, 0.9% saline; 3-5 μl) was injected (1 mm deep) into the left (ipsilateral to the lesion) superior colliculus (SC) in 6 adult cats (2-intact; 2-neonatal-lesionals; 7-DOA; 2-adult-lesioned). 48 h later the animals were procured for combined TMB/DAB histochemistry and the visual cortex was examined for retrograde labeling in 100 μm thick, coronal, frozen sections taken every 400 μm with counts made in every third section. Intact animals showed no evidence of a bilateral projection. Adult hemispherectomized cats showed only a few cells (<10) restricted to the anterior-most aspect of the 17-18 border. In contrast, neonatal lesioned animals showed as many as 95 cells located throughout the anterior and posterior extent of areas 17 & 18. These results indicate that following neonatal cerebral hemispherectomy the intact visual cortex projects bilaterally to the SC and suggests that this may underlie the partial sparing of the visual field. (Giannini Found., USPHS HD-05958, ROI NS25780, HD-06412).

527.19


The cell distribution of the corpus callosum (CC) projection in the rabbit is exuberant at birth and its tangential extent fills most of the medio-lateral area. This exuberant projection retracts during normal development to become restricted to the 17/18 border in adults. We have previously shown that disruption of normal activity of the visual system during development results in an increase in the tangential extent of the CC cell distribution in the adult rabbit. This increase is associated with the effects of altered activity by rearing animals in scotopic illumination. Seven Dutch-Belted rabbits were raised under strobe-illumination (8 Hz, 10 μsec flash duration) for 24 hours/day beginning on day 5. After five weeks, multiple injections of HRP (Sigma VI, 20% in H₂O) were made (12 μl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. Strobe-rearing resulted in a significant increase in the density of the CC cell distribution compared to the CC cell distribution in normally reared adult rabbits, although the tangential extent of the CC projection was normal. The results suggest that synchronous activation by retinal ganglion cells induced by strobe light may enhance the maintenance of callosal projections in the normal callosal zone, while patterned visual experience eliminates exuberant projections outside the normal callosal zone. Supported by grant NS26899.

527.21

CORPUS CALLOSUM CONNECTIONS IN VISUAL CORTEX OF NEONATAL CAT EXAMINED BY DOUBLE-LABELING WITH A CARBOXYFLUORESCENCE DYE (DIL) AND FLUORESCENCE IMMUNOREACTIVITY, D.J. Elberqer, Anatomy & Neurobiology, Univ. Tennessee Memphis, TN 38103.

DIL can be applied to aldehyde-fixed tissue to label neuronal elements without altering the tissue, and is visualized with a fluorescent light source using a filter appropriate for rhodamine. To define corpus callosum neurons within visual cortex of the neonatal cat, and to identify axons ending in the contralateral hemisphere of callosal neurons or of neurons in contact with callosal neurons, DIL-labeled tissue was subjected to immunohistochemical procedures. Cristals of DIL were placed in the midsagittal corpus callosum in slices of neonatal cat brain to label callosal cell bodies and processes. After several weeks in the dark, slices were cut, and incubated with an FITC anti-DIL antibody. Sections were mounted on glass slides and viewed for both DIL and neurotransmitter label. Antiserum labeled cell bodies and axon processes, and DIL-labeled labeling pattern. DIL-labeled callosal cells were double-labeled with antiserum to GLU and SS but not BAH. In some cases, antiserum-labeled neuronal elements were apposed to DIL-labeled callosal elements. Double-labeling with DIL and immunofluorescence provides a sensitive method for studying neonatal tissue. Supported by EY06362 and BSRG-RRO2492.

527.18


In adult rats visual callosal cells in layers II-Va of the cortex are distinctly concentrated at the border between areas 17 and 18. The distribution of visual callosal cells is fully mature as early as postnatal day (PND) 12. However, features of the mature pattern are speicified at earlier ages. A better understanding of the factors responsible for specifying the callosal pattern, we examined the distribution of callosal cells in the visual cortex during the first week of life. Bulk injections of a persistent, retrogradely, and fluorescent tracer (rhodamine-conjugated latex microspheres) were made into the PND 1, 3, and 7, and injected pups were allowed to survive for various periods of time prior to perfusion. Injections were aimed at the white matter, to ensure that all cells with an axon crossing the callosum were labeled. Assessment of the radial and tangential organization of callosally labeled cells in these pups indicates that adult-like features of the mature callosal pattern are present as early as PND 1. Cells that send axons across the callosum on PND 1 have completed their migration into the cortical plate and lie in the deep layers, in their final radial positions. The radial distribution of callosal cells is mature by about 6 days of age -- approximately a week prior to the tangential distribution. It is possible that maturation proceeds more slowly in the tangential plane because of factors that are extrinsic to the developing neocortex.
527.23
REORGANIZATION OF VISUAL CALLOSAL PROJECTIONS AFTER EARLY THALAMIC LESIONS IN THE GOLDEN HAMSTER. B. Howard*.
Cornell University, Ithaca, New York, 14853.
Various manipulations have been shown to preserve early exuberant callosal projections, including cortical damage and unusual visual environments. During early development the entire tangential extent of the visual cortex projects through the corpus callosum to the contralateral hemisphere. The mature callosal projection is restricted to regions near the border between primary and secondary visual cortex. Here we test whether visual callosal projections originate from a greater tangential extent when thalamic input has been removed from the contralateral visual cortex.

On the day of birth hamster pups received unilateral electrolytic lesions to the visual nuclei of the thalamus. The dorsal lateral geniculate nucleus (LGD) and sometimes the lateral posterior (LP) and lateral geniculate nucleus were damaged. At 30 days of age the animals received a unilateral implant of HRP in the thalamic ipsilateral to the damaged thalamus. The distribution of retrogradely labeled cells in the contralateral cerebral hemisphere was plotted. These animals were compared to normal adult animals with comparable HRP injections.

An increase in the tangential extent of callosally projecting cells in striate cortex was observed in every animal with early thalamic damage. Thus, thalamic damage, like other manipulations which alter patterns of neuronal distribution or activity, can modify the organization of callosal projections.

Supported by NIH ROI NS19245.

528.1
NEUROPEPTIDE K KINHIBITS PROGESTERONE-INDUCED LH SURGE IN ESTROGEN-PRIMED OVARIECTOMIZED RATS. S.P. Keira, M.G. Delbe* and A. Safa, Dept. OB-Gyn, Univ. Fla., Gainesville, FL 32610.
Neuropeptide K (NPK), a novel 36 amino acid tachykinin has been localized in various sites of the rat hypothalamus including those areas previously implicated in the control of gonadotropin secretion. NPK receptors also have been visualized in the rat brain. Intracerebroventricular (icv) NPK promptly suppressed LH secretion in ovarycized (ovx) rats to the range seen after estrogen treatment. These studies suggested that NPK may act as an inhibitory neuropeptide. We have examined the effects of icv and peripheral (ip) injections of NPK on the LH surge induced by progesterone (P) in estradiol benzoate (EB)-primed ovx rats. One week after implantation of icv cannulae and ovariectomy rats were primed with EB (30 µg/kg) at 1000 h (day 0). One day 1 rats received intracerebral cannulae and on day 2 blood samples were withdrawn at 1000 h before P injection (2 mg/kg) and at hourly intervals from 1000 to 1800 h for LH measurements by RIA. Either one injection of NPK (0.5 or 1.25 nmol in 3 µl saline) at 1000 h just before the onset of LH surge or two injections at 1300 and 1500 h inhibited the P-induced afternoon LH surge which occurred in control rats receiving icv saline only. However, icv NPK (0.5 or 1.25 nmol) at 1300 h failed to suppress the P-induced LH surge. Finally, to assess the specificity of NPK action, the effects of neurokinin A (NKA), another member of the tachykinin family comprised of a 10 amino acid sequence which is structurally similar to NPK, were studied. NKA injection (1.25 nmol, icv) at 1300 h produced partial suppression of the LH surge (3.5 nmol). These studies suggest that NPK acts centrally to inhibit the steroid-induced LH surge and it is relatively more effective than NKA. The profound inhibitory effects of NPK on LH release in ovx and steroid-primed ovx rats imply that hypothalamic NPK may serve as a mediator of inhibitory feedback effects of steroids on LH release. (Supported by NIH HD 08634).

528.2
GALANIN STIMULATES LH SECRETION FROM ARCULATE NUCLEI- MEDIAN EMINENCE FRAGMENTS IN VITRO: INVOLVEMENT OF AN a ADRENERGIC MECHANISM. F.J. Lopez*, and A. Negro-Hilar.
Galantin (GAL), a peptide distributed in the gut and brain, is strongly localized to regulate hypothalamic pituitary function. We evaluated the effects of rat GAL on LH and PEG2 release from arcuate nucleus-median eminence median eminence (MnME) explants. Bicarbonate buffer, LH and PEG2 levels in the medium were measured by RIA. The addition to the medium of rGAL (0.5 or 100 µM) increased the release of both LH and PEG2 in a concentration-dependent manner. The B28 was approximately 55 nmol and 80 nmol for LH and PEG2, respectively. GAL-induced LH and PEG2 release are coupled, since inhibition of PEG2 synthesis with indomethacin (10 µM) completely blocked GAL-induced LH release. In addition, an active catecholaminergic input was needed for obtaining the stimulatory effect of rGAL, since the addition of the a-adrenergic blockers, phentolamine or prazosin, impaired the ability of rGAL to release both LH and PEG2. In summary, rGAL stimulates LH and PEG2 release from AN-ME fragments in vitro in a dose-dependent fashion. Such an effect is blocked by both indomethacin and a-adrenergic blockers, indicating that rGAL stimulation of LH secretion requires activation of a-adrenergic receptors and involves PEG2 as an intracellular mediator.
FOLLOWING EXPOSURE TO GAMMA-AMINOBUTYRIC ACID (GABA) agonists, baclofen (GABAß agonist), or neither (control), was observed in female rats. H. Bergen* and D.W. Pfafß. Rockefeller Wis. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison. Wl 53715. GABA could act by (a) decreasing the electrical activity of the cell and the release of LHRH; (b) by decreasing LH secretion. Since the onset of puberty is characterized by increases in pulsatile LHRH release, it is possible that developmental changes in the NE neuronal system contribute to the increases in LHRH release associated with puberty. In contrast, vehicle infusion resulted in no change in LHRH release. To our knowledge, this is the first demonstration that in the long-term OVEX model, there was a marginal increase in mean levels of LHRH which may represent an inhibitory phase was observed. Despite considerable between-animal variation in mean levels of LHRH, there was a consistent effect of administration of 3-PG, and in the long-term OVEX rats. There was also a marginal increase in mean levels of LHRH which may represent a change in the LH releasing hormone (LHRH) secretion. Although indirect evidence strongly supports a role for IP in the feedback action of E2 on LHRH secretion, it is possible that developmental changes in the NE neuronal system contribute to increases in LH release associated with puberty. In order to determine whether increases in LH release or NE receptor dynamics occur during puberty, we tested the effects of MTX on LHRH release in prepubertal (10-20 mg/kg) and peripubertal (24-48 mg/kg) monkeys. Using an in vivo push-pull perfusion method on conscious monkeys, perfusates were collected on ice in 2 min fractions for 12 h. After 2 h of control sampling, MTX (10 or 100 mg/kg) or vehicle was infused for 10 min through the push cannula at 1-1/2 h intervals. LH levels in perfusates were estimated by RIA. LH release during the 20 min periods before and after MTX or vehicle administration was compared. Results: MTX increased LH release 6-fold in prepubertal monkeys, and 2.5-fold in peripubertal monkeys. In contrast, vehicle infusion resulted in no change in LH release. To our knowledge, this is the first demonstration that LHRH release during the morning period in E2-treated animals compared to the morning period of control animals. In E2-treated animals there was a suppressed pulse frequency of 3-PG and protein content of pulse duration of 3-PG in the afternoon compared to morning. These results suggest that an E2-induced alteration in 3-PG pulse frequency is inversely associated with LH release and may mediate steroid feedback action of E2 on LH release.

METHANOL (MeOH): An axonal degeneration agonist, stimulates STIMULATES IN VIVO LHRH RELEASE IN PRE- AND PERIPUBERTAL FEMALE RHESUS MONKEYS. A. Goei & E. Terasawa. Neurosci. Training Program & West. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, Wl 53715. The push-pull perfusion technique has been used extensively in our laboratory to measure the release of luteinizing hormone releasing hormone (LHRH) in conscious ovariectomized female rhesus monkeys. In most cases, LHRH release from the stalk-median eminence consists of pulses of uniform amplitude with a sharp rising phase, superimposed on a stable baseline level of LHRH. These pulses typically have an interpulse interval of 40-50 min and a duration of approximately 30 min, with peak levels being attained within 10-20 min. In a few cases, though, peaks of similar duration and latency were separated by prolonged interpulse intervals of 2-3 h. In contrast to the spontaneous LHRH pattern observed in 4 of the 19 monkeys, it was marked by a dramatic increase in LH release. To our knowledge, this is the first demonstration that LHRH release during the morning period in E2-treated animals compared to the morning period of control animals. In E2-treated animals there was a suppressed pulse frequency of 3-PG and protein content of pulse duration of 3-PG in the afternoon compared to morning. These results suggest that an E2-induced alteration in 3-PG pulse frequency is inversely associated with LH release and may mediate steroid feedback action of E2 on LH release.

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS FOLLOWING EXPOSURE TO GAMMA-AMINOBUTYRIC ACID (GABA) agonists. R. Norgoe and D. W. Pfafß. Rockefeller Univ., New York, N.Y. 10021. Studies (i) GABA appears to exert an inhibitory effect on LHRH neurons in the preoptic area (POA) (Lamberta et al., Exp. Brain Res. 25, 356-1975). GABA could be (a) decreasing LHRH gene expression or LHRH synthesis, or (b) decreasing the electrical activity of the cell and the release of LHRH. We study by (a) by measuring (b) by LHRH agonists in vivo and observing whether changes occur as determined by LHRH immunocytochemistry. Mature female rats were ovariectomized and posterior cannulae were implanted just dorsal to the POA. Ten days later an inner cannula, containing either THG (GABA agonist), baclofen (GABA antagonist), or neither THG, was inserted into each guide cannula. After 6 or 24 h the rats were sacrificed, perfused and LHRH examined by LHRH immunocytochemistry. Neither THG nor baclofen decreased the number of LHRH-ir cells observed, as compared to controls. In fact, there was a trend toward increased staining intensity of cells exposed to THG (but not baclofen). These results suggest that exposure of LHRH cells to GABA agonists does not directly reduce peptide levels, so that electrical activity decrease may comprise the primary mechanism of their neuroendocrine effect.

EXCITATORY AMINO ACIDS (EAA) stimulate LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) secretion from arcuate nucleus-medial eminence fragments in Vitro. A. Donoso, F. J. Lopez*, and A. Negro-Vilar. Reproductive Neuroendoc. Sec., Lab. of Mol. and Integrat. Neurosci., Neurosci., IHES, Rsl. Trl., Park, N. C. 27709. We evaluated the role of EAA on LHRH secretion using agonists or antagonists acting through different subtypes of glutamate receptors. Arcuate nucleus-medial eminence (AN-ME) fragments were prepared from male rats using the push-pull perfusion technique. LHRH was measured in vitro in a Krebs-Ringerbicarbonate (KRB) buffer and release of LHRH was measured by RIA. Glutamic acid (GLU) enhanced LHRH release from the nerve terminals at 10-50 min. The EAA agonists kainic (KA) and quisqualic (QA) acids at [10 M] evoked a 2-fold increase of LHRH release over basal levels. N-methyl-D-aspartic acid (NMDA) was inactive at a wide range of doses (from 0.05 mM to 5 mM). The threshold doses for the stimulatory effects of both KA and QA were decreasing by increasing the K+ concentration (14 mM) in KRB which induced a minor depolarization of the terminals. The range of minimal effective doses was KA [0.075 mM]/QA [0.1 mM]. NMDA was inactive at 10 mM. All the conditions tested, even in the presence of high K+ or Mg2+-free Medium. The selective antagonist of KA/QA receptors, DNX, blocks the KA stimulated release, whereas the NMDA selective antagonist APV had no effect on KA and GLU responses. These findings suggest that EAA stimulates LHRH release from AN-ME terminals acting on non-NMDA glutamate receptor sites.
528.11 LUTERINIZING HORMONE RELEASING HORMONE RESPONSE IN VITRO TO y-AMINOBUTYRIC ACID. G.A. Disjoe, N.J. McArthur and P.G. Haugen. Dept. An. Sci. and Vet. Anat., Texas A&M Univ. Coll. Sc., TX 77843. 2411. GABA has been proposed to be a regulator of LH release. Hypothalamic tissue, from adult male and ovariectomized (OVX; 3 to 4 weeks) rats, was placed in 12 x 75 mm polyethylene test tubes. Each tube contained 0.6 ml of Krebs-Ringer bicarbonate medium (10 mM glucose, 1% bovine serum albumin and 0.01% gelatin) and incubated for 2 hours at 37°C under an atmosphere of 95% O2 and 5% CO2 in a mixer vessel. At 30, 60, 90 and 120 min, 0.5 ml of media was removed from each tube (samples 1-4 respectively) and replaced with fresh media. Release of LH-RH (pg/30 min) into the incubate was determined by RIA and all values expressed as the difference between samples 3 and 0; mean difference = MD. Generally, control tissues released less LH-RH in the 3rd sample than the 2nd resulting in negative values. Tissues treated with 10^-7 M GABA or GABA analogues were similar. Male rat medial basal hypothalamic (MBH) with attached infundibulum (INF) released increased concentrations of LH-RH when treated with 0.1 mM (3.9± 2.5/12). [MnCl2 (30 µM)] but not 10^-6 M GABA. [C] compared to control (C), -1.6± 1/5. LH-RH release from OVX rat tissue with GABA was not different from control. Tissues treated with a mixture of GABA and GABA analogues were always increased. The mechanism by which melatonin exerts an anti-gonadotropic activity remains unresolved. Accordingly, we used acute in vitro incubations of melatonic hypothalamic tissues to investigate effects of melatonin (MEL) on hypothalamic GnRH neurons. At 1000 h (0.5 h after light onset), addition of 10^-7 M MEL, but not 10^-6 M, MEL to the incubation media decreased (p<0.05) GnRH release from individual median eminences (n=15/trtment), whereas at 1500 h none of the MEL concentrations significantly altered GnRH release (n=15/trts). In contrast, at 1000 h both 10^-7 M MEL, but not 10^-6 M, MEL increased (p<0.05) GnRH release from medial basal hypothalamic (MBHs, n=10/trtment), which contains the arcuate nucleus. Results of experiments with median eminences (suppression of GnRH release by MEL at 1000 h but not 1500 h) are consistent with evidence that MEL inhibits dopamine (DA) release in the a.m. but not p.m. When the arcuate nucleus was included, MEL-induced suppression of DA release resulted in decreased en-...
1. The text is a scientific paper discussing various aspects of ischemia and its effects on energy metabolism and ischemic models.

2. **EFFECTS OF NALOXONE ON LHRH RELEASE AS ESTIMATED BY INTRA-HYPophysial MICRODIALYSIS**

3. Microdialysis was used to determine if pituitary LHRH levels are changed by treatment with naloxone (NAL), an opiate receptor antagonist that has been shown to stimulate LHRH release at hypothalamic sites (Karahalios and Levine, 1988). Our goals were to determine if LHRH responses to NAL measured by intrahypophysial microdialysis resemble hypothalamic responses to NAL, and to assess opioid inhibitory tone in intact and castrate rats. To estimate LHRH levels, CMA/10 microdialysis probes (Carnegie Medicin) were selected to obtain every 5min for up to 11h. At 2, 5 and 8h, rats received NAL (2.5 or 10 mg/kg i.v.) or saline. In 5 animals (3 castrate and 2 intact), 2.5mg/kg NAL increased mean plasma LH (12±2.1 vs 6.3±1.7 ng/ml). Conversely, in another 5 animals (3 castrate and 2 intact), NAL significantly increased mean plasma FSH (12±2.1 vs 6.3±1.7 ng/ml).

4. **ISCHEMIA: ENERGY METABOLISM AND ISCHEMIC MODELS**

5. Intracellular high energy phosphates (HEP) were monitored in rat hippocampal slices by 31P NMR under conditions of continuous perfusion, no flow, and reperfusion, to model changes which occur during cerebral ischemia and reperfusion injury. Hippocampal slices (350-400 µm thick) were prepared from adult male Sprague-Dawley rats (100-300 g; Charles River, MA). Slices were incubated in a medium containing potassium chloride (100 mM) and glutaconate (100 mM) instead of glucose. The slices were maintained in a humidified atmosphere of 95% O2 and 5% CO2. At the end of the incubation period, the slices were transferred to a perfusion chamber and perfused with a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5% CO2. The slices were then exposed to anoxia (0 min) followed by 60 min of reoxygenation (21% O2).

6. **INTERACTION BETWEEN PENTOTHAL AND NALOXONE ON THE ACTIVITY OF THE HYPOTHALAMIC LHRH PULSE GENERATOR IN THE RHEUS MONKEY**

7. N.M. Grosser, C.I. Williams, K.T.O'Byrne, M. Nishihara, and E. Knoblo. Laboratory for Neuroendocrinology, University of Texas Health Science Center, Houston, TX 77225. Neuroendocrinology of midline structures (MILA), a synchronized with the pulsatile secretion of LH, can be recorded from the medial basal hypothalamus. Ovariectomy (OVX) prolongs the pulse interval of LH secretion (E2) after NAL treatment; this effect. Naloxone administration (1 mg/kg) to ovariectomized rats increased the LH pulse frequency and duration from 4.5±0.5 to 8.8±0.7 min (p < 0.001; n=5 experiments), suggesting that endogenous opioids may be involved in mediating the effect of E2. In later studies done in unanesthetized intact animals, however, naloxone had no effect (control 3±1.0 min; E2-treated 2.7±0.3 min; E2+naloxone 3±1.0 min; n=6 experiments). These results invalidate our original conclusion based on anesthetized animals and indicate an interaction between pentobarbital and naloxone towards the functioning of this hypothalamic pulse generator. They also emphasize the need for caution in interpreting effects of opioid peptide inhibitors on LH secretion in vivo.
**529.3**

**DISTRIBUTION OF CREATINE KINASE ACTIVITY IN GERBILL HIPPOCAMPUS BEFORE AND AFTER ISCHEMIA.**


Diet. of Oral Biology and Div. Neurosurgery, CRUW Schools of Dentistry and Medicine, Cincinnati, OH 45229.

We have previously shown that neural function is maintained for longer periods of ischemia when phosphocreatine is increased prior to the ischemic insult. The distribution pattern of CK activity is critical in the gerbil hippocampus. Adult male mongolian gerbils are grouped as follows: Control (no ischemia), post-ischemic groups (2, 3, 4 and 10 days after 5 min bilateral common carotid artery occlusion). The post-synaptic neural elements are gradually lost in area CA1 during the reflow period, allowing separation of CK activity from pre- and post-synaptic elements. Twenty-micron frozen brain sections are prepared and visualized at 4 μm samples are dissected from fiber tracts, synaptic regions and cerebral cortex layers in the dentate gyrus and areas CA1 and CA3. Tissue sections are assayed for CK activity by standard histochemical fluorometric methods.

**529.4**

**SYNAPTOSOMAL RESPIRATION DURING NEUROLOGICAL DETERIORATION FOLLOWING ANOXIA.**

*K. S. Wagner, T. Kleinholz, and R. W.*

Neurosci. Res. Services, CWRU, Case Western Reserve, Cleveland, OH 44106.

We have previously demonstrated that 100-μ thick brain slices show a decrease in mitochondrial respiration during the ischemic period. In this model, recovery was directly proportional to the time of the ischemic event. For a 10 minute episode no cells were lost, for 55 minutes 40% of the cells were lost. These results show that an anoxic insult reduces hypoxic-ischemic infarction and did not develop independently of lactic acid accumulation and coma during the hours following reperfusion. These results suggest that inhibition of pyruvate dehydrogenase kinase may facilitate a more rapid return to aerobic energy production, thus improving outcome.

**529.5**

**EFFECTS OF ANOXIA AND AGLYCEMIA ON A THICK HIPPOCAMPAL SLICE MODEL OF ISCHEMIA.**


Depart. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794 and Dept. Oral Biology, Case Western Reserve, Cleveland, OH 44106. (SP: J. HALPERIN)

We have previously demonstrated that 1000-μ thick brain slices show increased brain slice glucose utilization (SGU), lactate, and inulin exclusion spaces but retain relatively normal histology and ultrastructure, even at the slice center. (Newman et al., JCBFM, 8:586, 1988) In this study, we attempted to separate the effects of acidosis and energy depletion by studying four groups of slices subjected to 15 min insults: 1) Control (pO2 715, 4 mM lactate); 2) Anoxia (pO2 715, 4 mM lactate, 37°C); 3) AGLYCEMIA (pO2 715, 4 mM acidosis); 4) ANOXIA-AGLYCEMIA (pO2 715, 4 mM acidosis + Lac 400 μmol/kg). Slices were then transferred to another chamber pre-equilibrated with one of the three deprivation buffers for 15 min or removed and returned to the same chamber for the remainder of the experiment. Following recovery was determined, aminoglycoside K-R slices were removed for measurement of brain slice glucose utilization (SGU, JCBFM, 8:586, 1988, Newman et al., this meeting) after 5 min, 1 hr or 4 hours. SGU was quantified in eight regions of each slice.

SGU increases significantly in stratum radiatum (sRAD) of CA1 and CA3 compared to standard 540-μ hippocampal slices. In CONTROLS, sRAD SGU declines by about 40% over 4 hours, consistent with our suggestion that the 1000-μ brain slice models the ischemic penumbra. Slices exposed to AGLYCEMIA or ANOXIA-AGLYCEMIA show a decline of 75% over 4 hours whereas ANOXIC slices show a decline of 85%. We also observe a dramatic and transient increase in SGU in CA1 and CA3 stratum oriens immediately after ANOXIA or ANOXIA-AGLYCEMIA but not after AGLYCEMIA. These results show that it should be possible to separate effects of acidosis from those of energy depletion without acidosis in this model system.

**529.6**

**DIFFERENCES IN ANOXIC AND ISCHEMIC STATES OF UNIDENTIFIED NIL-II-115 NEURIBLASTOMA CELLS IN HIGH DENSITY CULTURES AS STUDIED BY 31P NMR.**

*P. Glavin.*


Previous studies have shown that neural elements are gradually lost in area CA1 during the reflow period, allowing separation of CK activity from pre- and post-synaptic elements. Twenty-micron frozen brain sections are prepared and visualized at 4 μm samples are dissected from fiber tracts, synaptic regions and cerebral cortex layers in the dentate gyrus and areas CA1 and CA3. Tissue sections are assayed for CK activity by standard histochemical fluorometric methods.

**529.7**

**IN VIVO MICRODIALYSIS PERMITS DYNAMIC MONITORING OF METABOLITE CHANGES RESULTING FROM SPINAL CORD ISCHEMIA.**

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We have previously shown that neural function is maintained for longer periods of ischemia when phosphocreatine is increased prior to the ischemic insult. The distribution pattern of CK activity is critical in the gerbil hippocampus. Adult male mongolian gerbils are grouped as follows: Control (no ischemia), post-ischemic groups (2, 3, 4 and 10 days after 5 min bilateral common carotid artery occlusion). The post-synaptic neural elements are gradually lost in area CA1 during the reflow period, allowing separation of CK activity from pre- and post-synaptic elements. Twenty-micron frozen brain sections are prepared and visualized at 4 μm samples are dissected from fiber tracts, synaptic regions and cerebral cortex layers in the dentate gyrus and areas CA1 and CA3. Tissue sections are assayed for CK activity by standard histochemical fluorometric methods.

**529.8**

**POSTHYPOXIC GLUCOSE SUPPLEMENT REDUCES HYPOXIC-ISCHEMIC BRAIN DAMAGE IN THE NEONATAL RAT.**

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(See, Sepulveda, CA 91330; Dept. of Neuro., UCLA Sch. of Med., Los Angeles, CA 90024.)

We evaluated the effect of posthypoxic glucose supplement in a neonatal hypoxic-ischemic animal model. Seven-day-old rats underwent bilateral ligation of the carotid arteries followed by exposure to 8% oxygen atmosphere for 1 hr. The extent of hypoxic-ischemic brain damage was assessed histologically 72 hr later. Supplement of 10% body weight of 5.4% glucose immediately after the hypoxic exposure reduced the volume of necrotic cells in the brain to 37% of the control value, and attenuated ischemic damage in stratum and dentate gyrus. At the end of the hypoxic exposure, glucose was added to plasma and brain lactate was 10 mM and 9 mmol/kg, respectively. Glucose supplement produced a rapid rise of glucose in plasma to 25-30 mM and in brain to 5-6 mmol/kg over the next 2 hr. Lactate in both plasma and brain gradually fell toward the baseline level during the first hour of recovery regardless of posthypoxic glucose supplement. In this model, restoring brain glucose after the insult reduced hypoxic-ischemic infarction and did not raise brain lactate levels. These results illustrate the important role of glucose in neonatal hypoxia-ischemia and the fact that full corticai infarction can develop independently of lactic acid accumulation.
529.9

RELATIONSHIP BETWEEN PLASMA GLUCOSE (PG) AND INFARCT VOLUME FOLLOWING FOCAL CEREBRAL ISCHEMIA REPERFUSION. K.T. Y. Leong, H. Liao, M. N. Hikino, D. C. E. Hsu and W. C. Y. Neo. Dept of Neurology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA. We studied the influence of repeated episodes of PG on ischemic brain injury and the timing of the effect. Reperfusion following temporary occlusion of the right middle cerebral artery and both common carotid arteries results in a cortical infarct. The infarct volume (IV) is dependent upon the duration of the 3-vascular occlusion. In the first 2 hr after PG 34 g/kg, IV 1.8% was greater than those of rats fasted for 24 hr or fed rats treated with insulin (IH) (1.7 units/kg, IP 70 min before ischemia) (Table). Fasted rats receiving glucose during ischemia, (intra-ischemic PG 34g/kg) resulted in greater IV (118.5±67) than those with glucose loading immediately after ischemia (intra-ischemic PG 134±36; IV 183±53) or 3 hr post-ischemia (intra-ischemic PG: 137±35; IV 31±56). Results suggest that PG during ischemia, but not post-ischemic reperfusion has a significant effect on ischemic brain injury.

529.10


Infusion Glycem (mm) Echma Infarct Pre/Post Test Echma Infarct

Pre/Post Test

Glycem (mm) Echma Infarct

MCA-0 Gluc Gluc +1 +2 +3 (%) (Mean±SE)

Glu/Glu 12 20 20 20 10 16.7 ±1 9

Glu/Ins 8 20 5 14 3 8 37.5 ±6.7

Glu/Sal 13 20 12 8 7 8 30.8 ±2.3

Sal/Glu 13 8 15 20 20 10 15.4 ±1.5

Sal/Ins 13 8 18 10 8 0 10.0 ±3.4

529.12

DELAYED REPEATED CEREBRAL ISCHEMIA: BEHAVIORAL AND NEUROPATHOLOGICAL OBSERVATIONS. D. Wang*, D. Corbett* and S. Evans* (Sponsor: D.W. McKay). Faculty of Medicine, Memorial University, St. John’s, NL, Canada, A1B 3V6. Repetitive cerebral ischemic episodes are most commonly found in transient ischemic attack (TIA) and stroke patients. At present no animal model involving delayed repetitive cerebral ischemia has been used to study functional deficits produced by ischemia. Cerebral ischemia was induced in gerbils by a 10 min bilateral occlusion of the carotid arteries using a chronically implanted occluding device. Occlusion procedure was performed once a week for 3 weeks. The large increase in locomotor activity that resulted from the first occlusion gradually subsided over the next 4 test days. The second and third occlusions failed to further alter locomotor activity. Animals experiencing one ischemic episode appeared to have reduced cell loss with survival. Survivors that received postocclusion saline trended towards smaller infarcts versus those receiving glucose. Hyperglycemia after ischemia may aid in the development of drugs for treatment of TIA and stroke patients.

529.13

LONG TERM FUNCTIONAL IMPAIRMENTS FOLLOWING CEREBRAL ISCHEMIA IN THE GERBILL. D. Corbett and D. Wang*. Faculty of Medicine, Memorial University, St. John’s, NF, Canada, A1B 3V6. A brief period of cerebral ischemia produces a marked increase in locomotor activity which may be due to the ischemic animal’s inability to form a ‘spatial map’ of the test environment. If this is correct, the increase in locomotor activity should be a relative indicator of the degree of damage since ischemia causes severe damage of the hippocampus. Eighteen anesthetized gerbils were subjected to either bilateral carotid artery (BCL) occlusion for a period of 5 min (N=8) or sham surgery (N=10). The gerbils were tested in an open-field beginning 24 hrs after surgery and continuing each day for 10 days. The ischemic animals exhibited a large increase in locomotor activity compared to control animals (p<0.01, ANOVA). The activity declined over the 10 day test period but remained twice that of control animals. The CA 1 region of hippocampus was extended in ischemic animals. A brief period of ischemia produces a long lasting deficit in spatial mapping ability. The limited recovery may be due to either other brain regions involved in spatial mapping (e.g. prefrontal cortex).

529.14

SOMATOSENSORY EVOKED POTENTIALS (SEP) AND NEURONAL DEGENERATION AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS. K. Suzuki, H. Ijika, W. Young, Deps. of Neurophysiology, Physiology & Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016. Middle cerebral artery occlusion (MCAO) produces infarcts in the rat’s frontostriatal cortex. We took advantage of the orderly somatotopic arrangement of cortical motor function to study electrophysiological and morphological consequences of MCAO. Vibrissae (trigeminal), median and sciatic nerve stimulation produced robust responses localized to the lateral paretic (Pari), forelimb (FL) and hindlimb (HL) cortical regions. MCAO abolished cortical responses in 3 regions within minutes and diminished subtropical white matter responses. Pari SEPs did not recover in any rat while FL, HL SEPs recovered within an hour. FL and HL SEPs usually recovered within 30 minutes and exceeded preinjury levels by an hour. Rats with extensive infarcts did not recover SEPs in all 3 regions. We used the Fink-Heimer (FH) method to study degenerating axons in the brainstem and spinal cord of the rats. MCAO caused unilateral degeneration of pyramidal tract and trigeminal tract degeneration reflected infarct size. All rats had marked degeneration of corticaliafoulons to the trigeminal nucleus. FH staining revealed distinctive arachnoid glial neurons by 6 hours after occlusion, present in all cortical layers close to the infarct but localized to upper cortical layers II-III at 0.5-2.5 mm from the infarct. By 3-7 days, massive terminal degeneration appeared in peri-infarct cortex but neither the number nor distribution of arachnoid glial neurons progressed. Thus, the MCAOinfarct always involved Pari, encroached upon FL and seemed to effect HL regions. FH staining indicated degenerated spinal tract degeneration was often much greater than expected from MCAO infarct size. This may aid in the development of drugs for treatment of TIA and stroke patients.
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529.15

SINGLE VESSEL RAT FOREBRAIN PERFUSION: ONE TECHNIQUE TO STUDY ACUTE ISCHEMIC AND/OR TRAUMATIC BRAIN INJURY. L. S. Pine, Dept. of Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

In the rat, forebrain perfusion can be reduced to that by one internal carotid artery without neurological deficit. This is done (for example) by occluding the left external carotid, right internal carotid, the distal basilar, both pyrargyral arteries, and both occipital arteries.

Total hemisphere ischemia is studied in the awake rat by reversible occlusion with a vessel loop of the left common carotid artery. Partial ischemia is studied when catheters are placed retrograde into the left external carotid, right internal carotid artery and the left common carotid artery is occluded and blood is pumped at a controlled rate from the femoral artery catheter through a perfusion pump and then into the hemispheres by way of the craniotomy.

We studied closed head diffuse axonal injury: the carotid loop is tightened in the awake rat. It lapses into decerebrate coma and the injury is then introduced. The carotid loop is then released and hemispheric flow is restored. (Note that the pharmacologic anesthesia is neither as deep nor as quickly reversible as in decerebrate coma as described.)

All surgical manipulations as described are easily tolerated by the rat. Cerebral injuries are introduced soon after the surgery with the rat in decerebrate coma. Regional perfusion, histologic and neurologic outcome studies will be presented.

529.16

DEVELOPMENT OF A REPERFUSIBLE MODEL OF PHOTOCHEMICALLY INDUCED THROMBOTIC STROKE IN THE RAT MIDDLE CEREBRAL ARTERY (MCA) TERRITORY. B. H. von Treskow, F. Martin, T. A. Norrega, Dept. of Neurology, Univ. Miami School Medicine, Miami, FL 33101.

A reproducible and reversible model of stroke induced by occlusive arterial thrombosis in situ has not been reported previously. Occlusion can only be done by irradiation with an argon laser beam interacting with intravenous photosensitizing dye. With rose bengal, a pure Type I (singlet oxygen) generator, irradiation with the laser beam alone (290 lines/mm荫) is composed entirely of agglutinated platelets. Lack of fibrin content precludes re-endothelialization with tissue plasminogen activator (t-PA) and tissue thrombolytics (TPH), presumed to be a Type I (free radical) generator and 457.9 nm irradiation an unstable red clot forms; fibrin content is implied by net fibrinogen increase and immuno-PAGE. To enhance the free radical pathway, norbixin, a singlet oxygen (but not superoxide radical) quencher, was injected to a blood concentration of 13.4 μM, and followed by PMN at 1.34 nm. The MCA at the level of the olfactory tract was exposed and irradiated with 4 μM of 457.9 nm light dispersed longitudinally by a Ronchi ruling into 3 beams. Occlusion occurred within 10 min, yielding 3 colorless clots stable for 1 hr against saline injection. Upon bolus intracarotid reperfusion within 10 min was observed, which was stable for times up to 1 hr.

This model may find utility in controlled studies of reperfusion injury.

529.17

CEREBRAL BLOOD FLOW DURING REPERFUSION AFTER FOCAL ISCHEMIA. W. R. Selman*, R. C. Crumrine*, K. A. Seta*.

R. A. Ratcheson, and W. D. Lust. Lab. of Experimental Medicine, Univ. Missouri-Columbia, Columbia, MO 65212.

TO STUDY ACUTE ISCHEMIC AND/OR TRAUMATIC BRAIN ISCHEMIA. W. R. Selman*, R. C. Crumrine*, K. A. Seta*.

Alterations in blood flow during early stages of reflow may be an important factor in the evolution of brain damage following focal ischemia. Spontaneously hypertensive rats (SHR) were anesthetized and the left middle cerebral artery occluded with a snare ligature for 1, 2, or 4 h. Cerebral blood flow (CBF) was determined by the [14C]iodoantipyrine technique in the ischemic limb after 15 min in after clip removal. The mean CBF in the contralateral cortex of 9 SHR was 159 ± 13 ml/100g/min. Marked decreases in CBF were evident in both the striatum and cortex medial to the ischemic focus. CBF in the cortex was 48, 41 and 63% of control after 1, 2, and 4 h of ischemia, respectively. In contrast, CBF in the ischemic region was only slightly elevated (i.e., 110% of cont) during reflow. In both the perifocal and focal cerebral cortex, the changes in CBF appeared to be independent of the ischemic duration. Although the hyperemia observed during reflow after global ischemia was absent, it would appear that reperfusion triggers a shunting of CBF from the perifocal region to the affected area following focal ischemia.

529.18


The feasibility of impedance imaging (Applied potential tomography, APT) of cerebral ischemia with epicortical or scalp electrodes has been assessed in an animal model. Cerebral ischemia was produced in anaesthetized rats by occlusion of the internal or common carotid arteries for 5 min. Impedance was measured at 50 kHz by a vector electrode method with Ag/AgCl discs on the cortex or wires in the scalp, spaced 2mm apart. Impedence increased on the cortex during occlusion by 29.2% (n=10 rats) and 7.9% (n=23 in 10 rats) and 2.7±2.1% in the scalp (n=23 in 12 rats). Similar changes were obtained with wider electrode spacings. The scalp increased by 0.9±0.8% in recordings at ambient temperature, but increases of 1.4±0.7% were still seen when the scalp temperature increased to 40°C. The increase in impedance was due to external warming. Similar increases (3.9±1.9%, n=12 in 4 rats) occurred when the scalp was surgically excised and then replaced before recording. These changes exceed the sensitivity of 0.1% impedance change of a prototype APT device (Brown, R. E., Barber, D. C. & Saagar, A. D., Clin. Phys. Physiol. Meas., 6:109-121, 1985). The scalp changes do not appear to be due to changes in temperature or other local scalp conditions. This suggests that APT could generate maps of stroke, and work in progress to assess its accuracy in this model.

529.19


Breakdown of blood-brain barrier occurs following cerebral ischemia. The exact time sequence and parenchymal distribution of extravasated serum components are not clear. We therefore studied the distribution of serum albumin in gerbil brains after unilateral carotid occlusion for 10 min and subsequent reperfusion using light and electron microscopic immunocytochemistry. By light microscopy, no reaction was observed until 6 hrs after reperfusion and it was still weak and limited to the neuropil and a few neurons in the subiculum-CAl region. A consistent reaction was observed for albumin in the neuronal perikarya or neuropil in the subiculum-CAl and medial CAI region until reperfusion for 24 hrs and in the dentate molecular layer until 48 hrs. Electron-dense precipitates were observed in the degenerated neuronal perikarya after reperfusion for 24 hrs. Our study suggested that albumin leaked into the brain parenchyma from vessel lesions after re-establishment of reperfusion and accumulated not only in the astrocytes but also in the degenerated neuronal perikarya and dendrites.

529.20

MATHEMATICAL MODELING OF HYDROGEN CLEARANCE BLOOD FLOW MEASUREMENTS IN PERIPHERAL NERVE. T. D. Lagerlund and P. A. Low, Neurology Department, Mayo Clinic, Rochester, MN 55905.

The hydrogen clearance method of blood flow measurement often yields biexponential wash-out curves. In peripheral nerve, which does not contain two discrete anatomical compartments, it has been suggested that arterio-venous shunt vessels may clear hydrogen, leading to the fast component of a biexponential curve. To assess this hypothesis, we simulated the wash-out of hydrogen from nerve tissue in the vicinity of a shunt vessel by modeling diffusion of hydrogen to the vessel and its transport by blood, in addition to removal by capillary exchange. We calculated the hydrogen tension in each compartment as a function of radial distance from the vessel, axial distance along the vessel, and time. This was done for various values of parameters appropriate for rat sciatic nerve, and the resulting clearance curves were fit to a biexponential curve to determine the fast and slow clearance rates and the relative weights of the two components. The results show that the clearance rates and weights are affected by all model parameters including arterial clearance rate, capillary flow, blood velocity in the shunt vessel, distance between vessel and tissue and Capillary flow to the clearance rates and weights.

FRIDAY AM

ISCHEMIA: ENERGY METABOLISM AND ISCHEMIC MODELS

1345
$29.21$ EFFECT OF ISCHEMIA AND REPERFUSION IN VIVO ON ENERGY METABOLISM OF RAT SCIATIC NERVE. P. J. Zollman*, K. K. Ward*, P. J. Zochodne*, J. D. Schmelzer*, J. D. Schraelzer, J. D. Zochodne. Department of Neurology, University of California, Los Angeles, CA 90024. We examined the energy state of the sciatic nerve in vivo using high resolution 2D spectroscopy to determine if energy state recovery occurs with reperfusion. The sciatic nerve was dissected from anesthetized rats and placed in a tissue chamber where nervous activity could be measured. The sciatic nerve was then exposed to 30 min of ischemia followed by 30 min of reperfusion. Energy state recovery did not occur with reperfusion, indicating that there is an irreversible loss of nerve energy state with ischemia.

$29.22$ A POSSIBLE NEUROPATHOLOGICAL MODEL FOR FETAL HYPOXIA. Y. Shen, F. Heid*, W. P. Smotherman and R. L. Iacono. Department of Psychology and Center for Developmental Psychology, SUNY Binghamton, New York 13901. Using a method developed by Smotherman and his associates for the study of preterm rat fetuses without discomfort to the mother, we undertook to evaluate the effects of transient umbilical cord compression on oxidative and metabolic pathways as indicated by cytochrome oxidase histochemistry and the later development of behavioral capacities. The cord was compressed for 0, 2, 6, or 12 min in different groups of fetuses which were killed immediately after the clamping for histochemical analysis. The other half were delivered by cesarian section and cared for by a newly parturient female. The pups were tested at P3 for righting reflexes and for the ability to avoid falling from an elevated platform. The clamping of the cord produced a reduction in cytochrome reaction product that varied with the duration of the clamping. Regional differences in the amount of change in the cytochrome reaction product were noted. Behavioral consequences of the intervention included longer latencies for the righting response and poor performance on the elevated platform. These results suggest that this model may be useful for evaluating the neural and behavioral consequences of umbilical cord compression behavior.

$29.23$ DIFFERENTIAL EFFECTS OF HYPOXIA VERSUS ANOXIA IN ANIMALS DEVELOPING R.A.T. EEG ACTIVITY. F. E. Turner, C. S. Applegate, D. Holtzman, and J. D. Schmelzer. Children's Hospital, Boston, MA 02115. Exposure to hypoxia and/or ischemia during the perinatal period is associated in human infants with a high risk of subsequent neurologic deficit. It is not well known how the immature brain differs from adult in response to hypoxia/ischemia. We compared acute in vivo EEG responses of rats exposed to varying degrees of O2 deprivation in development to that of adult Sprague-Dawley rats. In contrast, ischemia for up to 3 h in vivo did not alter EEG, CP, or glucose and did not increase lactate. Ischemia with reperfusion times of 3 to 3 weeks did not alter any of the metabolites. The fact that energy metabolism remains intact in vivo under conditions which consistently result in complete cessation of the nerve impulse, suggests that nerve impulse extinction in vivo cannot be explained on the basis of energy substrate depletion in mammalian nerve. Presumably, the minimal residual perfusion is adequate to maintain energy stores, but inadequate to maintain impulse transmission.

$29.24$ THE HYPERGLYCEMIC HORMONE GALANIN BLOCKS THE GLUTAMATE MEDIATED ANOXIC DEPOLARIZATION IN CA3 HIPPOCAMPAL NEURONS. by Y. Ben-Ari, M. Lazdunski*. INSERM U29, Hôpital de Port-Royal 123 Bld de Port-Royal, 75014 PARIS.

Galanin is a hyperglycemic hormone which inhibits insuline secretion from pancreatic B cells by activating K+ channels normally blocked by intracellular ATP (ATP-K+ channels). These channels are blocked by sulfonylureas which are widely used as antidiabetic drugs. High affinity binding sites to the potent sulfonylurea glibenclamide (Glib) have been found in the brain notably in the hippocampus (Mourre et al, Brain Res. 1989 (486) 159). We now report that the depolarization induced by anoxia in CA3 neurons is increased by Glib and blocked by galanin. Brief anoxic episode (1.5-3 min.) induced in CA3 pyramidal neurons recorded from slices, a large depolarization followed upon reoxygenation by a post anoxic hyperpolarization due to the reaction by oxygen of the Na+ intracellular pump. Anoxic depolarization is due to an enhanced release of an excitatory amino acid. Anoxia was blocked by tetradotoxin (1 μM) or kynurenete (1 mM). Bath application of Glib (2.5 μM) or galanin (1 μM) during the anoxic episode respectively augmented and blocked the anoxic depolarization. Both agents had little effect in oxygenated solution suggesting that their action may be mediated by ATP-K+ channels. We suggest that by blocking the toxic release of glutamate during anoxia, agents such as galanin may be important to prevent neuronal death during and following anoxia.

$29.25$ CHLORDECONE EFFECTS ON FOOD INTAKE AND GASTROINTESTINAL MOTILITY IN THE MALE RAT. J. Williams, B. Montagner, G. M. S. Galceran, D. H. Urban, University of Southern California, Los Angeles, CA 90002. Chlordecone, a man-made organochlorine compound, is known to interfere with regulation of eating behavior and intestinal motility; therefore, this study was undertaken to determine the effects of chlordecone on food intake and gastrointestinal (GI) motility. Adult male CD-1 rats were used for all experiments. Significant decreases in food intake and body weight were seen following treatment with 75 mg chlordecone/kg. However, a greater percentage of ingested food was retained in the stomach. A significant delay in movement of ingested food through the GI tract was observed. After ad lib. overnight, weight loss after food removal in animals treated with chlordecone was statistically significant from that of control animals only during the time period when treatment activity was at the lowest level. In conclusion, chlordecone treatment decreases food intake and movement through the GI tract, suggesting that chlordecone may affect the peripheral serotomeric system. Weight loss seen in chlordecone treated animals may be due primarily to decreased food consumption rather than motor activity. (NIH ES03351 grant to LL)
530.3

We have evaluated the effects of three inhibitors on esterase activities preferentially sensitive to two specific esterase inhibitors. We examined the effects of three inhibitors on esterase activities and specific activity. The results showed that the inhibition of esterase activities was retained. The Bolton-Hunter reagent gave the best results: a pseudo-first order association rate constant of 300 s⁻¹ and a dissociation constant (Kd) of 0.5 nM. The results showed that the inhibition of esterase activities was retained 50% or more of its native toxicity and retained the ability to bind to rat brain synaptosomes. The pseudo-first order association rate constant and dissociation constant are in keeping with the data of this study. The results showed that the inhibition of esterase activities was retained 50% or more of its native toxicity and retained the ability to bind to rat brain synaptosomes. Thus, the Bolton-Hunter reagent resembles that of unlabelled toxin.

530.5

We have evaluated the neurotoxic effects of aluminum (Al) on fetal rabbit midbrain explants using an in-vitro matrix system. Midbrain was explanted at 24 days' gestation and maintained in DMEM-P10 medium with 10% rabbit serum. After 15 days, cultures were treated thrice weekly with aluminum maltol (AM) for 24 or 25 days. Argyrophilic neuronal cell bodies, neuronal swellings, and perikaryal inclusions were observed in cultures treated with 11, 13 and 15 µM AM. In 1, 2 and 3 experiments respectively, compared with controls, ultrastructural and neurochemical changes were observed. Al-treated cells showed an accumulation of aluminum in the nucleus within a few days, and by day 5 many cells started to die. Cellular content of aluminum was reduced significantly by adding DFO (0.02%) in the media for 5 days, and many cells started to extend the cellular processes. It appears that DFO treatment reverses the effects.

530.7
INHIBITION OF RAT SERUM ESTERASE ACTIVITY. J.G. Chambers* S.L. Hartgraves and M. Murphy. Brain Res. Lab. of Biochem. The University of Texas at San Antonio, San Antonio, TX 78284.

Using the substrates p-nitrophenol-acetate and -butyrate, we examined the effects of three inhibitors on esterase activities of rat serum. Reaction mixtures contained in a final volume of 100 µl, 0.1 M phosphate buffer, pH 8.0, 2.5 mM substrate and 10 µl serum. Reactions were carried out at 37°C for 5 mins and terminated by addition of 600 µl cold 1:1 (v/v) chloroform-methanol. Partitioning of liberated phenol was facilitated by addition of 900 µl 2.78 M NaCl to the reaction mixture followed by centrifugation of 1.0 ml of the aqueous phase (upper) was removed and partitioned phenol converted to phenolate anion by treatment with 0.1 M Na2HPO4. A 500 µl aliquot of the aqueous phase (upper) was removed and partitioned phenol packaged in a sterile glass coverslip contained in plastic petri dish. Cells were first grown in media that contained 0.05% aluminum tetrachloride for 5 days, then changed to the aluminum-free media. Cells were then exposed to aluminum (1, 2, or 3 µM) for 24 hours. Cellular content of aluminum was detected by morrin stain, and cellular processes were measured on photographs taken every 24 hours. The control neuroblastoma cells extended long cellular processes with many branches. However, the majority of the aluminum-treated cells did not extend processes; further, those cells that extended processes were characterized by short processes that were rarely branched. Most of the aluminum-treated cells showed an accumulation of aluminum in the nucleus within a few days, and by day 5 many cells started to die. Cellular content of aluminum was reduced significantly by adding DFO (0.02%) in the media for 5 days, and many cells started to extend the cellular processes. It appears that DFO treatment reverses the effects.

530.8

Hydrogen sulfide (H2S) is a toxic. H2S causes death by paralysis of central respiratory drive. Few neurochemical studies have been done to elucidate its toxicity. Using the push-pull perfusion technique, we studied the effect of H2S on the release of amino acid neurotransmitters in a brainstem nucleus responsible for maintenance of respiration and cardiovascular homeostasis. Recovered perfusates were separated and quantified by HPLC. In vivo, H2S (10mg/kg, IP) increased excitatory (GLU & ASP) and inhibitory (GLY & GABA) neurotransmitter release pattern. It caused, however, a delayed decrease in the release of GABA and glutamate. We observed that the release of glutamate to 61±2.2% of control (p<0.05, n=5). NSH (2µg/ml, close to endogenous H5Slevels) applied directly into the nuclei, by 1 min. produced no change in excitatory or inhibitory transmitter release, but 10µg/ml produced a delayed decrease (p<0.05 compared to basal; n=5) in release of glycine to 61.9±7% of control. Since glycine is the main inhibitory neurotransmitter in brainstem and spinal cord, decreases in its release may represent disinhibition leading to unopposed excitatory events and loss of respiratory drive. (Supported by Occupational Health & Safety Heritage Grant; SBK is an NRC( Canada) Student).
530.9
HYDROGEN SULFIDE INHIBITS RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS AND SYNAPTIC POTENTIALS IN VITRO: MECHANISM OF TOXICITY? R.J. Baldessar, R.J. Reffenstein and W.F. Colmers (SFON: W.M. Warren), Department of Pharmacology, University of Alberta, Edmonton, AB, CANADA, T6G 2H7

H2S has a variety of toxic effects, including psychiatric reactions, amnesia, and paralysis of central respiratory drive. We have studied the mechanism(s) of H2S action on hippocampal slice, in vitro rat olfactory bulb and in vivo rat olfactory bulb. Inhibitory hipppocampal slices (450 µM) were prepared using standard techniques, and held at 35°C in oxygenated buffer at 2 µM. Two pyramidal cells were impaled with 2M K+ acetate-filled microelectrodes (85-150 µA). NafH2 (40-160 µM) was dissolved in buffer just prior to application via the bath. Brain acid-soluble S2- is 75±M at the pH7.5.

NafH2 (10 µM) caused a significant (P<0.05), concentration-dependent hyperpolarization of CA1 cells, and a further, concentration-related hyperpolarization was often observed just after washout (P<0.05 at 160 µM). These changes were seen in low CaCl2, high Mg2+ medium, which abolished synaptic transmission, and were often associated with a decrease in membrane input resistance. EPSP's evoked by stimulation of stratum radiatum were significantly (P<0.05) attenuated by NafH2 at concentrations ≥ 60 µM. 160 µM NafH2 reduced the EPSP by 17±3% of control. All effects of NafH2 were rapidly reversible (5 - 15 min).

Results suggest that inhibition of neuronal activity by H2S (e.g., retrograde amnesia) may involve suppression of synaptic and/or direct hyperpolarization. Similar mechanisms may be operative in the brainstem, and account for the loss of central respiratory drive seen in acute intoxication. 

Supported by MRC (Canada) and Alberta Occupational Health and Safety Heritage grants; NS has an MRC (Canada) Studentship.

530.11
POSTNATAL DEVELOPMENT OF CATALASE ACTIVITY IN THE OLFACTORY PATHWAY. R. Coopersmith and M. Leon. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Olfactory receptor neurons, located in the olfactory epithelium and olfactory' bulb. Catalase metabolizes hydrogen peroxide to water and oxygen, preventing peroxidative cellular damage. Catalase activity was assayed in olfactory epithelium, olfactory bulb and olfactory cortex. In all three tissues, catalase activity increased during the first week of life, peaking at PND 10, when activity was slightly more than twice that on PND 1. Enzyme activity then declined over the next five days, below PND 0 levels. In bulb and cortex, activity remained low until PND 40, with bulb activity consistently higher than cortex. Catalase activity in olfactory epithelium steadily rose until activity on PND 40 was slightly higher than on PND 0. The small but consistent difference between bulb and cortex levels may be a result of very high activity in the nerve layer, comprising olfactory receptor axons, which represents a relatively small proportion of the entire bulb volume.

530.12
INCREASE IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN OLFACTORY PATHWAYS IN RESPONSE TO FORMALDEHYDE. M. Miller, R. Coopersmith, and M. Leon. Deparment of Psychobiology, University of California, Irvine, CA 92717.

Dendritic processes of olfactory receptors lie in the nasal mucosa, the protection of the blood-brain barrier making them more easily exposed to environmental toxicants than the rest of the brain. Since it seems that olfactory pathways may be a route for entry of toxicants into the CNS, we examined catalase activity in olfactory pathways, as a possible protective mechanism in this system. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the hexose monophosphate shunt, shows unusually high activity in olfactory neurons. The hexose monophosphate shunt produces NADPH required for cytochrome p450 and glutathione detoxification systems. If the hexose monophosphate shunt is involved in detoxification in olfactory pathways, it might respond to an airborne toxicant such as formaldehyde.

Rat pups were exposed to formaldehyde (1:1 dilution of saturated vapor) for 1 hour/day from postnatal days 10 - 20. G6PD activity was then measured in exposed and control rats at postnatal day 20. Exposed rats showed higher G6PD activity in olfactory epithelium and olfactory bulb, while activity in cortex was the same for control and exposed rats. This finding suggests that detoxification systems in olfactory pathways may be modified in response to environmental challenges.

530.14

Amphotericin B methyl ester (AME) retains antifungal activity but is less nephrotoxic than AB. However, the association of AME with diffuse white matter degeneration has prevented its continued clinical use. The neurotoxicity of these compounds was studied by subperineural injection (10 ul) of AB or AME (1 mg/ml), or saline, into the rat sciatic nerve using a glass micropipette; fixed tissues were examined between 1-14 days. In AME-injected nerves, reactive axonal swellings with distal axonal atrophy (suggesting axostasis) and myelin bubbles were more present at 1-4 days; by day 2, all tissues were affected. In AB-injected nerves, somatofugal degeneration, less regenerating sprouts and remyelinating axons were evident at 7 days. AB produced similar changes, albeit with more pronounced degeneration, less regenerating sprouts and remyelinating axons. At 14 days, most fibers demonstrated somatofugal (proximal) axonal atrophy. In AME-treated nerves, this axotomy-like response was more prominent than axonal loss and may arise via an alteration in trophic mechanisms due to impaired axonal transport and/or demyelination-induced conduction block. (Supported by NS26265).
351.1

PROCYCLIDINE PROTECTS AGAINST SOMAN NEUROTOXICITY, EVEN WHEN ADMINISTERED AFTER ONSET OF CONVULSIONS. N.L. Proc. G.A. Swann and W.M. Swann. School of Medicine, St. Louis, MO

Soman, an organophosphate cholinesterase inhibitor, induces a devastating neuromuscular syndrome featuring persistent muscle weakness and widespread spread of muscle paralysis. The primary problem in studying this agent is posed by the marked individual variation in sensitivity of experimental animals. Some adult rats develop spastic motor reflexes only minutes after receiving a dose of soman in the range of 30-125 µg/kg and many rats typically succumb to severe brain damage and die, yet others can tolerate much higher doses without experiencing seizures, brain damage or death. Administration of diazepam to soman exposed rats that had not received diazepam before soman was administered consistently, increased the percentage of animals susceptible to soman neurotoxicity.

In the present study, we evaluated the ability of procyclidine to protect against soman neurotoxicity. Procyclidine is an anti-Parkinsonian drug with anticholinergic properties which also antagonizes the N-methyl-D-aspartate-(NMDA) receptor (a subclass of glutamate receptors hypostatically implicated in seizure-induced brain damage). The individual variability problem was obviated by the following research design: all animals were pretreated with LiCl (5 mg/kg ip) and 24 hr later given soman (125 µg/kg) and observed for symptoms; animals that began convulsing were treated immediately either with saline or a single dose of procyclidine (75 mg/kg ip). All animals were killed 4 hours after soman treatment and their brains examined histologically. Rats that did not secrete (n = 28) did not have any brain pathology. All rats that received procyclidine (n = 12), stopped seizing within 5 to 15 minutes; all of these rats escaped brain damage. When atropine (up to 100 mg/kg ip) was substituted for procyclidine in the above protocol, it conferred no protection against soman neurotoxicity. While it is unclear whether the preferential action of procyclidine can be best explained in terms of an anticholinergic or NMDA antagonist mechanism or (or both), it is significant that procyclidine provided soman neurotoxicity even when given after neurotoxic symptoms had become manifest. Supported by R01 NH 38894 (JW), ES 07060 and DAMD 17-86-C-6010.

351.2

LONG-TERM POTENTIATION IS REDUCED IN RATS PRETREATED WITH LOW DOSES OF SOMAN. D. L. Armstrong, T. Okada, S. A. Miller*, B. Z. Kerenyi* and R. B. Murphy. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78285 and USAFSAM, Brooks AFB. 

Rats exposed to sublethal doses of soman display a number of symptoms that include weight loss, dehydration, hypotension and hyperreactivity. In these experiments the magnitude and duration of long-term potentiation (LTP) within the dentate gyrus following tetanic stimulation of the perforant path was compared between soman-exposed and non-soman exposed animals. The amplitude of population EPSPs evoked by a test pulse was measured in urethane anesthetized rats before and after a series of tetanic stimulation trains were applied to the perforant path. Control animals (n = 5) displayed 100% increases over baseline EPSP amplitudes of 2.2 ± 0.4 mV (x ± S.D.) which were maintained for the duration of the three and a half recording sessions. Two to three weeks after soman exposure, experimental animals (n = 5) displayed much greater variability in baseline EPSP amplitudes and, either no potentiation or response to tetanic stimulation, or a small amount of potentiation (≥50%) occurred which was not maintained for the full three hours. Loss of dentate was observed in animals that had recovered from other symptoms such as hyperreactivity. These results indicate that disruption of physiological mechanisms associated with learning and memory functions occurs in animals exposed to low doses of soman. Supported by Air Force Contract No. F33615-87-D-0909.

351.3

A CENTRALLY ACTING NOVEL TERTIARY PYRIDOSTIGMINE DERIVATIVE 3-(N,N-DIMETHYLCARBAMOXYL)-1-METHYL-4- TETRAHYDROPIRIDINE (THP) IN COMBINATION WITH PYRIDOSTIGMINE PROTECTS AGAINST SOMAN TOXICITY. E. E. Clark*, K. W. Ford*, R. B. Knight*, L. W. Harris* and J. A. Broomfield*. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21005-5425.

Reversible inhibition of acetylcholinesterase (AChE) in both central and peripheral compartments may be required for protection against soman intoxication. We report that earlier (FASEB J. 3(3):A899, 1989) that THP, in penetrates the blood-brain barrier and inhibits AChE in both blood and brain, whereas pyridostigmine (Pyr), an irreversible inhibitor of AChE in the blood only. Here we report that in pretreatment of guinea pigs with THP (262 µg/kg) plus Pyr (131 µg/kg) 30 min prior to acetylcholine challenge, up to 100% antimuscarinic or oxime treatment protects 60% of the animals against 2xLD50 of soman. Inhibition of AChE in the brain (150% of baseline) and the blood (150% of baseline) is required prior to soman challenge, with no protection against soman neurotoxicity. It is significant that procyclidine prevented soman neurotoxicity even when given after neurotoxic symptoms had become manifest. Supported by R01 NH 38894 (JW), ES 07060 and DAMD 17-86-C-6010.

351.4


Soman is an irreversible, organophosphate (ACSE), organophosphate neurotoxin. Using the degradation-sensitive cupric-silver histological method of deOlmos, damage to the central nervous system (CNS) from both acute and chronic low doses of soman has recently been associated with chemical unprotected rats (Sci. Neurol. Abstr. 14(1):174). In this study we sought to detect CNS damage using the cupric-silver method in rhesus monkeys that were also evaluated for motor performance deficits (Gluck et al., these proceedings). All monkeys were pretreated with pyridostigmine via minipump and received an acute dose (3xLD50, im) of soman while provided with protective therapy of atropine and 2-PAM (an AChE reactivator). One half of the animals also received diazepam as an antioxidant. The animals were sacrificed after 1 or 14 weeks. In the first two monkeys examined, extensive degeneration was found in the non-diazepam treated monkey, after one week, that was comparable to the damage we have observed in rats. The areas affected include: amygdala, piriform cortex, hippocampus, select thalamic nuclei and subpopulations of cortical aeras. Brain stem, hypothalamus and upper spinal cord were spared. In the diazepam treated monkey no damage was detectable after 14 weeks of survival. Supported by USAF Contract F33615-87-C-0625 with funding from the USAMRICD.

351.5


In 12 rhesus monkeys well trained on the Primate Equine Platform (PEP) task, we tested performance recovery after exposure to 2 times the estimated ID50 of soman on days 1, 2, 3, and 6 following exposure, and weekly thereafter, for 4 weeks. All subjects received pyridostigmine before, and atropine and 2-PAM after soman exposure; half of them also received diazepam after soman.

Diazepam was administered to reduce the duration of soman-induced convulsions. Survival was more likely in diazepam-treated subjects (89%) than in non-diazepam treated monkeys (50%), but this difference was not statistically significant due to the small n involved (p < 0.30, using χ2).

For animals that survived the 12 hr and 24 hr post-soman performance tests, diazepam conferred a significant relative protection against the soman-induced performance deficit. For animals that survived for the full 13 weeks of post-soman testing, diazepam treatment had no overall effect on performance, but tended to shift the performance deficit to a later period in time. Recovery of performance continued throughout the 13 weeks. Performed under USAF Contract F33615-87-D-0567/002 with funding from the USAMRICD.

4.5-DKT was inhibited by glutathione. In primary neuronal rising lymph potentiated the neurotoxicity of 4,5-DKT.

4.5-DKT showed dose-dependent toxicity. Glutathione, corresponding to alpha and beta subunits of G-protein, were compensated increase in 5-HT turnover and altered tryptophan metabolism. 4,5-DKT administration decreased 5-HT and its metabolites in the limbic system. Recent studies demonstrated lateral ventricle effect on basal 5-HT efflux in central 5-HT neurons.

DYN A showed no effect on striatal SP. DYN A showed increased levels of SP in the striatum. Fortidive dyskinesia (without dyskinesia) showed increased levels of SP in the striatum. DYN A showed no effect on striatal SP. DYN A showed increased levels of SP in the striatum.

Regional GAD activities were monitored in these brains as before. Decreased activity of glutamate decarboxylase (GAD) was found in the hippocampus with 285 umol min.mg (1/4 liver levels) followed by substantia nigra, striatum, and cortex with 80% of maximal value and cerebellum only 50%. In animals led enkephalin inducing drugs of 0.75%, 1-butyrylhydrazinol of PBDT, and 0.05% Sudan 1 only QRS was induced in the hippocampus, substantia nigra, and cerebellum. No induction of GST was observed for any brain region dissimilar to the usual induction of these enzymes in peripheral tissue by these inducers.

We speculate that the brain regional differences in levels of the protease enzyme-QR and GST during toxic challenge may be a determinant of selective neuronal degeneration.

The neurological effects of p,p'-DDT are thought to result from a delayed closing of the sodium channel following spike generation. In studies, p,p'-DDT was shown to inhibit female rat sexual behavior possibly as a result of enhanced transmitter release. The effects of p,p'-DDT on the serotonergic system was examined in cortex, hippocampus and hypothalamus. Proestrus female rats were treated with oil, 25 mg/kg or 75 mg/kg p,p'-DDT and were sacrificed that evening.

There was a dose-dependent decrease in 5-HT levels in cortex and hippocampus but elevations in 5-HIAA were present only in the hypothalamus and only at the higher dose of p,p'-DDT. When hypothalamic slices were perfused in vitro with p,p'-DDT, the compound produced an increase in serotonin release. 5-HT-P1-P2-P3 binding to the 5-HT1A site, examined in hippocampus and cortex, was complex. The dose of 25 mg/kg p,p'-DDT produced an increase in the Max for binding to cortical and hippocampal membranes. Females given 75 mg/kg p,p'-DDT showed a binding profile that was impossible to analyze by linear or nonlinear regression. The results of the in vivo and in vitro studies indicate a disturbance of the serotonergic system of female rats after treatment with p,p'-DDT. (NIH ES03351 grant to U)

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SYNAPTogenesis: neuromuscular junction

532.1 SYNAPTIC PLASTICITY IN SKELETAL MUSCLE: CHANGES IN THE GOLOI APPARATUS DURING DEVELOPMENT AND AFTER DENERVATION. (L. Larjava 1 & Cartaud 2, N. Pereyra 1 and J. Lezoualc'h 1, Institut Jacques Monod, CNRS, Paris 1, Centre Neurochimique, Guif-sur-Vette, 2 Institut Pasteur, Paris 2, France.

In the course of studies about the cellular and molecular mechanisms of synapse formation, we carried out an immunofluorescence study of the distribution of the Golgi Apparatus (GA) in skeletal muscle fibres and after endplate formation in 15-day-old chicks. Using a monoclonal antibody against GA, we confirmed the known distribution of GA in myogenic cells. We focused our study on the distribution of GA and nNOS in myogenic cells and cultured muscle fibres of innervated and non-innervated parts of the muscle. In denervated muscle fibres, we observed a decrease in the amount of GA and a decrease in the number of fibres with GA positive. In contrast, GA positive fibres were found in non-innervated parts of the muscle fibre. The GA positive fibres were found in the non-innervated parts of the muscle fibre. These results suggest that the distribution of GA in skeletal muscle fibres and after endplate formation in 15-day-old chicks is influenced by the presence of GA positive fibres and the presence of endplate formation.

532.2 REGULATION OF INTERSTITIAL CELLS AND MATRIX MOLECULES IN DENERVATED MUSCLE. E.A. Connor, Univ. of Mass. Amherst, MA.

Denervation of skeletal muscle results in a selective accumulation of interstitial cells in junctional regions. These interstitial cells, fibroblasts, may play a role in regeneration of neuromuscular junctions; fibroblasts may extracellular matrix which is directly involved in synapse formation. In fact the accumulation of interstitial cells in denervated frog muscle is accompanied by remodelling of the matrix environment. Denervated interstitial cells are displayed in the presence of the extracellular matrix and they exhibit a characteristic pattern of staining against fibronectin, N-CAM and tenascin, when compared to the pattern of stain in innervated muscles.

To determine the factors that regulate the appearance of the interstitial cell accumulation and the remodelling of the matrix after denervation, I have examined whether blockade of muscle activity is sufficient to initiate these denervation responses. Activity was blocked postsynaptically by α-bungarotoxin. Interstitial cell density was determined for functional and extrajunctional regions of muscles. In controls, the junctional cell density of denervated muscles was increased. In denervated muscles treated with α-bungarotoxin, the junctional cell density remained unaffected. Therefore, the functional accumulation of interstitial cells and matrix remodelling are not triggered simply by loss of muscle activity.

532.3 INTRACELLULAR CALCIUM LEVELS IMMEDIATELY AFTER NERVE-MUSCLE CONTACT. S.K. Young, S.H. Hulser, & A.D. Grinnell, Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

A report that contact of positively charged beads with cultured Xenopus muscle cells will produce transient changes in muscle cell calcium within seconds (Zhong & Feng, Dev. Biol. 128(1):61) prompted us to monitor intracellular calcium levels following nerve-muscle contact in these same Xenopus cultures.

Cells were manipulated into contact with the muscle cells at various points. The amount of calcium was measured immediately after the contact with a fluorescent calcium indicator. The calcium levels were found to be lower than those found in normal muscle cells. In contrast to experiments with beads, no large changes in calcium levels were observed after nerve-muscle contact. Supported by Grants from MDA, NSF, and NIH.


Sites of high acetylcholine receptor density at neurite-muscle contacts were stained with Fluorescent α-bungarotoxin and observed daily for up to 15 days. In cultures of embryonic spinal cord neurons and myotomal muscle cells, the great majority (90%) of these neurite associated receptor patches (NARPs) formed within 1 day of neurite-muscle contact. New NARPs continued to form as long as the neurites continued to grow and establish new contacts. Based on their length, intensity and area, NARPs are defined by their identity. In controls, the junctional cell density of denervated muscles was increased. In denervated muscles treated with α-bungarotoxin, the junctional cell density remained unaffected. Therefore, the functional accumulation of interstitial cells and matrix remodelling are not triggered simply by loss of muscle activity.

INTERACTIONS OF NEURONS WITH NORMAL AND VARIANT C2 MUSCLE CELLS IN CULTURE. M. T. Lupe*, H. Gordon, and Z. W. Hall. UCSF, Department of Neurobiology, San Francisco, CA 94143.

Myotubes of the C2 mouse muscle cell line were cultured with mouse spinal cord (SC) or chick ciliary ganglion (CG) neurons. We used immunofluorescence with alpha-bungarotoxin and antibodies to pre- and postsynaptic components to characterize the contacts between neurons and C2 myotubes. These contacts were then compared with those formed on two genetic variants of the C2 cell line, R', which synthesizes reduced amounts of acetylcholine receptor (AChR); and S27, which makes reduced amounts of sulfated proteoglycans.

Neurites of both SC and CG cells showed staining either with antibodies to neurofilament protein (NF) or to synaptic vesicle protein (SV), but usually not to both. In wild-type C2 co-cultures, myotubes were preferentially associated with SV-containing neurites as opposed to NF-containing neurites. This preference was also found in co-cultures with the R' variant, but was not found with S27, the proteoglycan-defective variant, which either showed no preference or a slight preference for NF-containing neurites.

Nerve-muscle contacts in wild-type co-cultures were characterized by AChR clusters that were associated with clusters of 43 kDa protein, as well as clusters of the extracellular matrix components, laminin and JS-1. Nerve-muscle contacts in R' co-cultures showed none of these specializations while those in S27 co-cultures did.

This work was supported by grants from the NIH and the MDA.

CONCANAValin A INHIBITS AGRIN-INDUCED FORMATION OF AChR, AChE, AND HSP AGGREGATES. K.W.K. Tsim* (spoon: U.J. McMahan). Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

Agrin, a protein isolated from Torpedo electric organ, induces the formation of aggregates of acetylcholine receptors (AChRs), acetylcholinesterase (AChE), and heparan sulfate proteoglycan (HSP) on chick myotubes in culture. In this study, concanavalin A (Con A) was used to examine the role of lateral migration in the formation of AChR, AChE, and HSP aggregates. Con A has been shown to immobilize AChRs and so prevent their redistribution. Con A also binds to AChE and so might prevent its lateral migration as well. Preincubation of chick myotubes with Con A inhibited agrin-induced AChE aggregation, as expected. Incubation with Con A also reduced the rate of dispersal of AChE aggregates after removal of agrin.

Adding A nerve fiber with agrin inhibited not only agrin-induced AChR aggregation, but also agrin-induced accumulation of AChE and HSP. Sucinyl Con A, which has little effect on the mobility of AChRs, also inhibited agrin-induced AChE and HSP pachytane. Con A inhibition of agrin-induced aggregation of AChE and HSP, but not of AChR, was reversed by increasing the amount of agrin added to the culture. These results suggest that (1) AChE does not accumulate in agrin-induced specializations by lateral migration and (2) that Con A not only prevents aggregation of AChRs by immobilizing them, but also prevents aggregation of AChRs, AChE, and HSP by acting as a competitive inhibitor of agrin.


Agrin, an acetylcholine receptor aggregating molecule isolated from Torpedo, is concentrated at the neuromuscular junction. We have previously shown that molecules antigenically related to agrin are localized at acetylcholine receptor clusters (AChRc) on aneural muscles in vivo. Here we report that muscle-derived agrin can be detected on the surfaces of cultured myogenic cells as early as 24 hours after plating and continues to accumulate for at least 2 weeks, as judged by immunofluorescence microscopy and radiolabeled agrin. Muscle-derived agrin is also expressed in cultures grown in serum-free media. A portion of the muscle-derived agrin colocalizes with spontaneous AChRc. Agrin-related molecules derived from muscle are also present at motoneuron-derived nodes of Ranvier in culture. Taken together, these results indicate that muscle-derived agrin, (perhaps in concert with agrin present in the culture medium), plays an important role in the organization of the postsynaptic apparatus at the neuromuscular junction.

AGRIN DOES NOT INDUCE ACHR CLUSTERS IN A VARIANT MUSCLE CELL. H. Gordon & Z. W. Hall. Dept. of Physiology S-762, Univ. Calif. School of Medicine, San Francisco, CA 94143.

Cultured myotubes of the C2 mouse muscle cell line spontaneously cluster acetylcholine receptors (AChRs) on their surface. We have isolated a variant subtype, S27, that expresses AChR on its surface but does not form spontaneous clusters of AChR. In co-culture of S27 with cells from mouse spinal cord or chick ciliary ganglion, AChR clusters form near sites of contact with neurites.

Agrin isolated from Torpedo electric organ has been shown to induce AChR clusters in chick and amphibian muscle cultures (Nitkin et al. (1987), JCB 105: 2471). We show here that agrin also induces AChR in primary cultures of mouse muscle and in cultures of the C2 mouse muscle cell line. Agrin does not induce clusters in the variant S27.

The failure of agrin to induce clusters in S27 suggests that the basis of neural clustering of AChR requires neural factors other than, or in addition to, agrin.

We thank B. Wallace and U. J. McMahan for their generous gift of agrin and for their advice.

TWO PATHWAYS FOR AGRin-INDUCED ACCUMULATION OF AChRs, AChE, AND OTHER POSTSYNAPTIC COMPONENTS. B. G. Wallace. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Agrin, a protein extracted from the electric organ of Torpedo californica, causes the formation of specializations on chick myotubes in culture that resemble the postsynaptic apparatus at the vertebrate skeletal neuromuscular junction. The specializations contain cytoplasmic (43 kDa AChR-associated protein), membrane (AChRs and acetylcholinesterase [AChE] and extracellular matrix-associated heparan sulfate proteoglycan [HSP] and A2 asymmetric AChE proteins. AChRs accumulate in agrin-induced specializations by lateral migration of receptors already in the myotube membrane at the time agrin is added. To determine whether other postsynaptic components accumulate by a similar mechanism, we examined the effects of inhibition of protein synthesis and secretion. We found that agrin-induced accumulation of AChRs and the 43 kDa AChR-associated protein is not blocked by inhibitors of protein synthesis or secretion, indicating that the 43 kDa protein, like AChRs, accumulates by redistribution of pre-existing molecules. Agrin-induced accumulation of HSP, AChE, and BuChE was blocked by inhibitors of protein synthesis and secretion, indicating that accumulation of these components does not occur by redistribution but requires insertion and/or release of newly synthesized proteins. Thus, different components of the postsynaptic apparatus accumulate in agrin-induced specializations by different pathways.

DENERVATION OF THE NEUROMUSCULAR JUNCTION CAUSES A REDUCTION OF AGRIN-LIKE MOLECULES AND ACHRG-AGGREGATING ACTIVITY IN THE SYNAPTIC BASAL LAMINA. A. L. Reis, Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

The portion of a muscle fiber's basal lamina that lies in the synaptic cleft of the neuromuscular junction contains molecules that direct the aggregation of acetylcholine receptors (AChRs) and acetylcholinesterase (AChE) on regenerating myofibers. Agrin, a protein extracted from basal lamina-rich fractions of the electric organ of Torpedo, contains these three molecules in several ways. For example: 1) agrins direct the formation of AChR and AChE aggregates on cultured muscle fibers, 2) AChE-aggregating activity is present in skeletal muscle and can be immunoprecipitated with monoclonal antibodies against agrin, and 3) agrin and agrin-like molecules stimulate neurotransmitter release from basal lamina preparations. A study described here examined the possibility that denervation causes a decrease in the synaptic basal lamina making the muscle fibers' basal lamina intact. New muscle fibers were allowed to regenerate within the basal lamina sheaths of muscles that had been predenervated for 4 weeks, at which time the muscles were damaged in such a way as to cause degeneration of all cellular elements at the neuromuscular junctions, while leaving the muscles' fibrous basal lamina sheaths intact. New muscle fibers were allowed to regenerate within the basal lamina sheaths of the original muscle fibers for 3 weeks while reinnervation was prevented. Control muscles were reinnervated at 3 weeks. The muscles were then stained for AChE to label the original synapse sites on the basal lamina sheaths and AChRs were labeled with 125I-bungarotoxin. The density of AChRs at former synapse sites in the regenerated muscles that had been predenervated was 42.9% of control regenerated muscles (p<0.001, student's t-test). Thus denervation for 4 weeks caused a 2.4-fold reduction in the synaptic basal lamina's AChR-aggregating activity.
VATED CULTURED HUMAN MUSCLE. V. Askanas. J. McFerrin.*

AChE stain is present only at the NMJs (noninnervated)

CHARACTERISTICS OF NEOSTRIATAL NEURONS TRANSPLANTED

E L E C T R O P H Y S I O L O G I C A L  A N D  M O R P H O L O G I C A L

excitatory post-synaptic potentials (EPSPs) in response to electrical

before grafting. Two-5 weeks after transplantation, the rats were sacrificed,

brains removed, and 400 µm thick slices through the transplant were taken

Grafted neurons had average resting membrane potential values of -46 ± 2.0

S

Glycoconjugate(s) in the extracellular matrix at NMJs

neuromuscular junctions (NMJs), we have examined PNA-BM

and AChR distributions in muscle/nerve cultures of

and both it and its receptors are found in brain.

The present study tested the hypothesis that IG-I

gene expression in muscle correlates with neuromuscular synaptogenesis in rats. In 70% of

were abundant about the time of birth, when

polyneuronal innervation is maximal. These

transcripts were down regulated with the same
time course as postnatal elimination of excess

synapses. Following nerve transection, adult

muscle IGF-I mRNAs were up regulated, correlating

with the renewed capacity of mature muscle to

accept re-innervation when denervated. These

data are consistent with a model in which (i)
elvated IG-I mRNA contributions to loss of polyneuronal innervation, (ii)
synaptogenesis provokes an inhibitory signal which is relieved upon nerve transection, and (iv) IG-I follows its mRNA levels.


of somata was 17.1 ± 0.9 M m. This study shows that neostriatal neurons can

be successfully grafted into the substantia nigra and form functional synaptic

connections between glucocorticoid and AChE inhibitors are commonly used.

To investigate the role of PNA-BMs in the formation of neuromuscular junctions (NMJs), we have examined PNA-BM and AChR distributions in muscle/nerve cultures of Xenopus laevis. PNA specifically recognizes glycoconjugate(s) in the extracellular matrix at NMJs in frog (Xo, 1987) and in Xenopus tadpoles as shown by rhodamine-PNA and fluorescein-α-bungarotoxin staining. In culture, PNA-BMs begin to appear within 2 days of plating muscle cells and generally colocalize with AChR clusters. However, in 2-3 day cultures, ACHR clusters outnumber and therefore are not always associated with PNA-BMs. Whereas in older (5-6 days) cultures, PNA-BM distribution increases and colocalization with ACHR clusters becomes more complete on both aneural and innervated muscle cells. Our results suggest that PNA-BMs appear after AChRs during synaptogenesis. How the distribution of basal lamina PNA-BMs might be regulated by nerve-muscle interaction during embryonic development is being investigated.


Insulin-like growth factor I (IGF-I) may play a role in the development of neural circuitry. For example, IGF-I stimulates neurite formation, and both it and its receptors are found in brain. The present study tested the hypothesis that IG-I gene expression in muscle correlates with neuromuscular synaptogenesis in rats. In 70% of muscles, IG-I mRNAs were abundant about the time of birth, when polyneuronal innervation is maximal. These transcripts were down regulated with the same time course as postnatal elimination of excess synapses. Following nerve transection, adult muscle IGF-I mRNAs were up regulated, correlating with the renewed capacity of mature muscle to accept re-innervation when denervated. These data are consistent with a model in which (i) elevated IGF-I mRNA contributions to loss of polyneuronal innervation, (ii) synaptogenesis provokes an inhibitory signal which is relieved upon nerve transection, and (iv) IG-I follows its mRNA levels.

532.13

INFLUENCE OF HYDROCORTISONE (HC) ON ACETYLCHOLINESTERASE (AChE) AT THE NEUROMUSCULAR JUNCTIONS (NMJs) OF INNERVATED CULTURED HUMAN MUSCLE. Y. Askunas, J. McFerrin.

G.C. Lee, U.K. Engel, Neuromuscular Center, University of Southern California School of Medicine, LA, 90017.

In human muscle cultured in monolayer and innervated by fetal-rat spinal cord with dorsal root ganglia attached, AChE stain is present only at the NMJs (noninnervated cultured human muscle is negative in AChE stain). Under our standard conditions, all AChE sites are linear and thin between 5-21 days of innervation; between 4-8 wks of innervation, 37% of them become complexly organized. Our laboratory has demonstrated functional synaptic connections between fetal-rat spinal cord with dorsal root ganglia attached, AChE is present only at the NMJs (noninnervated cultured human muscle is negative in AChE stain). Under our standard conditions, all AChE sites are linear and thin between 5-21 days of innervation; between 4-8 wks of innervation, 37% of them become complexly organized. Our laboratory has demonstrated functional synaptic connections between fetal-rat spinal cord with dorsal root ganglia attached, AChE is present only at the NMJs (noninnervated cultured human muscle is negative in AChE stain). Under our standard conditions, all AChE sites are linear and thin between 5-21 days of innervation; between 4-8 wks of innervation, 37% of them become complexly organized.

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533.3 DEVELOPMENT OF TYROSINE HYDROXYLASE (TH) POSITIVE NEURONS IN THE HUMAN EMBRYONIC SUBSTANZA FOVA (SN). I. I. Frese, T. S. Frese, J. A. Speier, J. M. Division of Neurosurgery, University of South Florida, College of Medicine, Tampa, Fl. 33612 and Hana Biolo-

There is current interest in the role of grafting human cadaver embryonic dopaminergic neurons into the striatum as a means of treating Parkinson’s disease. In rodent models, transplantation of neuroblasts at the time of donor tissue harvesting is critical. We have therefore examined the development of the human nigral dopamine neurons in the striatum in rats and non-human primates. These implications for neural grafting. T.J. CluJer, J.E. Springer, M.J. viability and plasticity as an adjunct to our ongoing studies of DA neuron grafts in studies include initial screening in monolayer cultures of fetal rat venơaα mesencephalon, that a factor derived from segments of adult rat sciatic nerve significantly enhances the the grafts of cultured fetal pig DA neurons, a marker for Parkinsonian rat model. R.D. Young, N. Hase, M.E. Spencer, S.H. Depts. Neurobiology and Anatomy and neurology, University of Rochester School of Medicine, Rochester, NY 14620.

We recently reported long-term survival and function of developed dopamine neurons from a rat model of Parkinson’s disease. Here we extend this finding by showing that grafts of cultured fetal pig DA neurons also function in this rat model. In addition, we have demonstrated that these grafts of dopaminergic neurons are proliferating in our cell culture system. Donor tissue was dissected from the ventral mesencephalon of 22-24 day fetal pigs, enzymatically dissociated, triturated, and kept in proprietary media formulations for 15 days. Typically, a 3 x 10^5 cells were plated per well and, after 6–10 days proliferation, the cells were transplanted (1 well/rat). The cultured tissue was injected directly into the DA-denervated striata of host rats. Eleven of 20 grafted rats have shown behavioral recovery in the amphetamine-induced rotation test. Preliminary histological analysis revealed large grafts containing numerous DA neurons (as identified by tyrosine hydroxylase (TH) immunohistochemistry). A parallel experiment combining immunohistochemistry and autoradiography has shown that when such cultures are labeled for 2 days in vitro with 3H-DA (days 5+) numerous TH-positive grafted neurons containing label are found at 4 weeks post-grafting.

533.4 FUNCTIONAL TRANSPANTATION OF PROLIFERATED FETAL PIG DOPAMINE (DA) NEURONS IN THE PARKINSONIAN RAT MODEL. R.D. Young, N. Hase, M.E. Spencer, S.H. Depts. Neurobiology and Anatomy and neurology, University of Rochester School of Medicine, Rochester, NY 14620.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989

533.5 DETECTION OF TYROSINE HYDROXYLASE mRNA IN TRANSPLANTED FETAL DOPAMINERGIC NEURONS. Y. Solberg, Y. Pollak and W. F. Silverman. Units of Morphology (YS & WFS) and Immunology & Microbiology, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, Israel. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of dopamine (DA). It is known that the gene, coding for production of this enzyme is strictly regulated during the differentiation process of a functional state of the DA neuron. We have examined the expression of the TH gene using the in situ hybridization technique in transplanted fetal substantia nigra neurons; adrenal medulla) is being studied as an experimental therapy for Parkinson’s disease. Most of these patients require pharmacological treatment with amphetamine. We have recently reported long-term survival and function of developed dopamine neurons from a rat model of Parkinson’s disease. Here we extend this finding by showing that grafts of cultured fetal pig DA neurons also function in this rat model. In addition, we have demonstrated that these grafts of dopaminergic neurons are proliferating in our cell culture system. Donor tissue was dissected from the ventral mesencephalon of 22-24 day fetal pigs, enzymatically dissociated, triturated, and kept in proprietary media formulations for 15 days. Typically, a 3 x 10^5 cells were plated per well and, after 6–10 days proliferation, the cells were transplanted (1 well/rat). The cultured tissue was injected directly into the DA-denervated striata of host rats. Eleven of 20 grafted rats have shown behavioral recovery in the amphetamine-induced rotation test. Preliminary histological analysis revealed large grafts containing numerous DA neurons (as identified by tyrosine hydroxylase (TH) immunohistochemistry). A parallel experiment combining immunohistochemistry and autoradiography has shown that when such cultures are labeled for 2 days in vitro with 3H-DA (days 5+) numerous TH-positive grafted neurons containing label are found at 4 weeks post-grafting.


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We have been investigating methods for enhancing embryonic dopaminergic (DA) neuron viability and neurite extension after transplantation. We have now developed a method of culturing DA neurons with adult rat sciatic nerve followed by co-grafting experiments in unilateral DA-depleted rats. We found that a factor derived from segments of adult rat sciatic nerve significantly enhances the viability of developing midbrain DA neurons. Cultures consisted of cell suspensions derived from day 13-16 embryonic pig substantia nigra neurons; adrenal medulla) is being studied as an experimental therapy for Parkinson’s disease. Most of these patients require pharmacological treatment with amphetamine. We have recently reported long-term survival and function of developed dopamine neurons from a rat model of Parkinson’s disease. Here we extend this finding by showing that grafts of cultured fetal pig DA neurons also function in this rat model. In addition, we have demonstrated that these grafts of dopaminergic neurons are proliferating in our cell culture system. Donor tissue was dissected from the ventral mesencephalon of 22-24 day fetal pigs, enzymatically dissociated, triturated, and kept in proprietary media formulations for 15 days. Typically, a 3 x 10^5 cells were plated per well and, after 6–10 days proliferation, the cells were transplanted (1 well/rat). The cultured tissue was injected directly into the DA-denervated striata of host rats. Eleven of 20 grafted rats have shown behavioral recovery in the amphetamine-induced rotation test. Preliminary histological analysis revealed large grafts containing numerous DA neurons (as identified by tyrosine hydroxylase (TH) immunohistochemistry). A parallel experiment combining immunohistochemistry and autoradiography has shown that when such cultures are labeled for 2 days in vitro with 3H-DA (days 5+) numerous TH-positive grafted neurons containing label are found at 4 weeks post-grafting.


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533.11


Female rats were lesioned with 6-OHDA 8 mg in 2 µl in the left substantia nigra. At least one month later they were tested with amphetamine 5 mg/kg s.c. and apomorphine 0.35 mg/kg s.c. A suspension containing approximately 1.5 x 10^8 cells from the ventral mesencephalon of rat embryos was distributed in three sites in a triangular fashion in the center of the denervated striatum. Grafted rats received no lesion and remained asymptomatic during the tests. The group with grafted striatum showed a decrease in rotational behavior in comparison with the non-grafted side.

533.12


Schallert and Hall (Behav. Br. Res. 30:15-24, 1988) have reported that rats with unilateral 6-hydroxydopamine (6-OHDA)-lesioned nigrostriatal tract that have recovered their rotation response to periorbital stimulation do not show a significant decrease in disengage behavior. The focus of the present study was to examine the effects of intrastriatal fetal nigral grafts on disengage behavior. The results showed that grafting led to a significant decrease in rotational behavior, suggesting protection for the lesioned rats.

533.13


We have previously shown (Houldoult, C. Neuropsychopharmacology. 26: 1501, 1987) that repeated administration of L-DOPA enhanced the rotation induced by the substantia nigra 6-OHDA lesion, which is associated with a progressive increase in the percentage of the response and the increase in the percentage of the response is more marked in the presence of the estrous cycle. The present study examined the effects of repeated administration of L-DOPA plus benzamidine in lesioned female rats. The results showed that repeated administration of L-DOPA plus benzamidine led to an increase in the percentage of the response, which was associated with a decrease in the percentage of the response.

533.14


Human fetal mesencephalic tissue, obtained following termination of first-trimester pregnancy, was grafted to the ventral striatum of previously unilaterally D-2-denervated rats. Dopaminergic neurones, originating from the graft, were identified by staining with dopamine-β-hydroxylase and tyrosine hydroxylase. Immunohistochemical and electron-microscopic observations indicated that the grafts contained a substantial number of dopaminergic neurones, which were distributed in the ventral striatum. The grafts were found to be well integrated into the host tissue, and dopaminergic neurones were observed in all regions of the grafts. The grafts were also found to be able to inhibit the expression of the denervated striatal dopamine receptors, suggesting that the grafts were able to compensate for the loss of dopamine receptors.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
SOLUBLE NEURITE PROMOTING ACTIVITY IN FETAL OR TERM AMNION M.A. PALMATIER, R.J. FLUNKETT* and L.L. KOPIN CNB and SHB, NINDS, NIH, Bethesda, Maryland 20892

The basement membrane of full term human amnion has been shown to be a permissive substrate for neurite outgrowth in vitro and in vivo. We have investigated fetal and term amnion as a source of soluble neurite promoting activity for use in animal models of Parkinson's Disease. Monkey and human fetal amnion and human term amnion was tested for soluble neurite promoting activity by incubating amnion with dorsal root ganglion (DRG) explants in serum-free medium. Neurite outgrowth from the DRGs was compared to neurite outgrowth from DRGs induced by various concentrations of NGF. All amnion tested induced neurite outgrowth from DRG explants. The neurite outgrowth is not blocked by antibodies to mouse NGF. Neurite outgrowth from DRG explants did not require contact with the amnion. During long-term storage of amnion in phosphate-buffered saline (PBS) at 4°C there is loss of neurite promoting activity from the amnion and appearance of neurite promoting activity in the PBS. These results suggest that amnion contains a soluble neurite promoting activity.

SOLID ADRENAL GRAFTS IN LONG-TAILED MACAQUES: STEROETEOIC IMPLANTATION AND BIOCHEMICAL STIMULATION. M. Dubach & D.C. German, Psychiatry and Behavioral Sciences and Regional Primate Research Center, University of Washington, Seattle WA; and Departments of Physiology and Psychiatry, University of Texas, Dallas TX.

Tissue-culture experiments have demonstrated the ability of biochemicals such as nerve growth factor and laminin to increase the survival and neurite production of adrenal medulla cells, depending on donor age and species. Here we present efforts to establish a reliable method for CNS transplantation of adrenal medulla in long-tailed macaques by including these substances in the medium during transplantation. In some cases grafts were further supplemented by chronic infusion of nerve growth factor into brain tissue or lateral ventricle. The physical preparation of graft tissue may also be critical for viability. Previous solid grafts have involved diced tissue preparations, but in the present experiments we have grafted intact strips of the medulla as a whole was separated from surrounding cortex and sliced into strips of equal thickness. Each strip was immersed in HBSS with or without biochemical supplement, loaded with its medium into a liga spinal needle, and stereotaxically implanted into dopamine-denervated caudate or putamen. Turning behavior of the unilaterally treated animals was monitored by rotometer. After 3-4 weeks, graft and brain tissue were examined by tyrosine hydroxylase immunohistochemistry. (Supported by USPHS grants NS25752, RR00666, and Dallas Area Parkinsonism Soc.)

TRANSPLANTATION: STRIATUM I

BEHAVIOR REVERSAL IN RAT MODELS OF PARKINSON'S DISEASE FOLLOWING GRAFTING OF FREEZING AND THAWING ADRENAL MEDULLARY TISSUE. G.F. Zhang*, O. Lu*, Y.S. Wang* and S.S. Jiao. Beijing Institute for Neuroscience, Capital Institute of Medicine, Beijing, 100054, China

In this study the feasibility of storing adrenal medullas at low temperature was investigated by freezing the morphology of graft cells and testing its function. The medulla grafts taken from adult Sprague-Dawley rats, freeze-dried, and stored at -196°C for one week, were transplanted into the caudate nucleus of host brain, where the unilateral substantia nigra was damaged by 6-OHDA. During the observation period of three months, the test for rotation behavior induced by apomorphine shows that the rotation reduced partly after operation, and that the longer the rat survived, the less the number of rotation reduced. Histological examination reveals that the shape of graft cells and the feature of staining are normal, and that abnormal tissue reaction and degeneration do not occur in host brain. Histofluorescent study shows the graft cells still maintain the function of secreting catecholamine. It is suggested that freezing to low temperature does not adversely affect the ability of adrenal medulla tissue to survive, and that by the low temperature frozen-storage method, the medulla grafts may be used for experimental research and clinical application. (Supported by NIH RR-86-230)


We have investigated the effect of systemic injection of MPTP and subsequent grafts of adrenal medulla into the striatum of young(2-3mo.) and aging(12mo.) mice. MPTP treatment (4X20 mg/kg i.p. given 3 or 12 hr apart) resulted in 80-90% depletion of striatal dopamine(DA) and disappearance of tyrosine hydroxylase(TH)-immunoreactive(IR) fibers in both young and aging mice 5 weeks after treatment, aging mice showed no apparent recovery. Adrenal medullary minced pieces were grafted i.e. the striatum of MPTP-treated young and aging mice. In young mice, dense TH-IR fibers were observed in the grafted striatum and the DA content on the graft side recovered to 40% of the normal level. In aging mice, receiving similar grafts, TH-IR fibers also were observed in the grafted striatum, but less dense and more restricted around the area of graft. Compared with young mice, DA content on the grafted side was 29% of the normal level. These observations indicate that the nigrostriatal DA system in aging brain could be recovered by adrenal medullary grafts, but the degree is more limited compared with young brain. Supported by NIH R37 AG06960 and a PDM Foundation Center Grant.
DECREASED ASYMMETRY IN STRIATAL EXTRACELLULAR Dopamine with behaviorally effective Adrenal medulla grafts increases turnover of dopamine and amphetamine-stimulated microdialysate dopamine concentration in an animal model of Parkinson's Disease. Results from this lab have demonstrated that adrenal medulla grafts increase brain DA turnover, and amphetamine-stimulated release without producing a decrease in CSF DA or basal extracellular concentrations of DA in striatum. The experiments reported here were undertaken to further define the mechanism(s) through which adrenal medulla grafts mediate recovery of function in striatum.

Adult male rats with unilateral 6-OHDA lesions of the substantia nigra and intranigral adrenal medulla grafts underwent in vivo microdialysis 6 months post-graft. Dialysis probes were placed in both the DA-denervated and intact striata. Basal concentrations of DA, DOPAC and HVA were determined 24 later in freely moving animals.

Graft-induced behavioral recovery (>10% decrease in APO-induced turning) was associated with decreased asymmetry in basal extracellular DA in striatum (p < 0.02, compared to animals with adrenal medulla grafts that did not decrease turning). The difference in asymmetry was due to decreased extracellular DA on the intact side rather than increased DA on the graft side. DOPAC and HVA concentrations were also decreased on the intact side (compared with control animals). Therefore, recovery of the response to APO may be associated with graft-induced compensatory changes in striatal DA activity of the contralateral striatum. (Supported by NIH grants NS522157 & NS81056).


We have employed a learned, visuomotorically-driven, 'cuckoo clock' reversal task to study co-grafting of autologous adrenal medullary tissue and sural nerve into the left caudate nucleus in 2 MPTP hemiparkinsonian macaques. In this stimulus-initiated task, reaction time is measured from a go-signal to movement onset (RT/MO) and movement time (MT), the physiological correlate of bradykinesia, is measured from movement onset to achievement of final target. Eight hemiparkinsonism induced by left intracarotid injection of 4 mg/kg of 6-OHDA/4 µl resulted in a prolongation of movement time and reaction time in both animals: Animal #1 - RT/MO 300 ± 10 (mean ± SEM in msec) and MT 110 ± 35 in normal state vs. RT/MO 591 ± 49 and MT 241 ± 24 eight months after MPTP; Animal #2 - RT/MO 210 ± 13 and MT 160 ± 12 in normal state vs. RT/MO 582 ± 72 and MT 2058 ± 73 one year after MPTP. Co-grafting was performed via a transcallosal intraventricular approach with direct implantation of adrenal medullary and sural nerve tissue fragments into the head of the left caudate nucleus. Preliminary results from animal #1 at 5-6 months after co-grafting reveal an improvement of MT (147 ± 33) and RT/MO (423 ± 29) compared to the parkinsonian state.


A major advantage of employing polylactide-co-glycolide for biodegradable controlled-release microcapsule systems within the CNS is the ability to modify the duration of drug release by manipulating the biodegradation kinetics of the polymer i.e. changing the ratio of lactide and glycolide in the copolymer. DA was microencapsulated in 2 different excipients. One is a 50:50 lactide/ glycolide copolymer (DA 50:50); the other is a DA 65:35 lactide / glycolide copolymer (DA 65:35). The 65:35 copolymer-being more water-soluble than the 50:50—promoted a longer release of DA. Male rats (Sprague Dawley) were unilaterally lesioned with 6-OHDA (8 µg/4 µl). When a stable baseline was present in culture, the DA microcapsules were implanted unilaterally in the striata of 6-OHDA treated rats. The results indicate that the microcapsules delayed the development of rotational asymmetry behavior was recorded for 2-3 hours. Implantation of DA 50:50 microcapsules elicited immediate contralateral rotation whereas there was a 60-90 minute delay of contralateral DA microcapsules following injection of DA 65:35. Amphetamine induced rotation was decreased by 50% up to 8 weeks in rats bearing DA 65:35 microcapsules. Implanted DA 50:50 microcapsules did not alter amphetamine-induced behavior. These results show that by modifying the polymer excipient it is possible to attain functionally significant amounts of DA in the CNS for prolonged periods of time by simple administration of microencapsulated DA.

BLOOD-BARRIER PERMEABILITY ASSOCIATED WITH ADRENAL MEDULLA GRAFTS. E.J. Curran and J.J. Becker. Neuroscience Program and Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

In rats with unilateral 6-OHDA lesions of the substantia nigra (SN), intranigral adrenal medulla grafts reduce amphetamine (AMPH)- and apomorphine (APO)-induced rotational behavior. The mechanism involved in this behavioral recovery is unknown. We hypothesize that increased permeability of the blood-brain barrier (BBB) adjacent to the graft allows dopamine to diffuse into the denervated striatum. Adult rats with unilateral SN lesions were tested for APO- and AMPH-induced rotational behavior prior to and after receiving intranigral adrenal medulla grafts or control operations in vivo microdialysis. Some grafts resulted in a decreased behavioral response to AMPH while others produced a decrease in the response to APO. Animals with a decreased behavioral response to AMPH had AMPH-induced DA release in striatum that was seen using microdialysis; when DA was injected into the jugular, penetration into the denervated striatum occurred with the average increase in striatal DA being from 9.45 ± 5 to 91.45 pg/µl. However, animals that showed a decreased behavioral response to APO and control animals did not show either the AMPH-induced DA release or the peripheral DA in the denervated striatum. These results show that: (1) an increase in BBB permeability is associated with APO-induced DA release and (2) decreased rotational asymmetry in rats with adrenal medulla grafts, (2) there may be more than one mechanism involved in mediating the different behavioral effects of adrenal medulla grafts. (Supported by NS22157).


The behavioral effect of sustained intrastriatal release of DA from a polymer matrix was analyzed in a 6-OHDA unilaterally lesioned rat model. Rods of poly(ethylene-vinyl-acetate copolymer) (EVA) containing 20% by weight of dopamine were pressure extruded and coated with several layers of pure EVA. Biocompatible semipermeable acrylic copolymer microcapsules were implanted subcutaneously in the flank of lesioned rats. Five animals received dopamine loaded rods, while controls received rods comprised of EVA alone. Two weeks postimplantation, unilaterally lesioned rats showed a decrease in their rotational behavior under apomorphine challenge. Two weeks after the removal of the dopamine-releasing rod, rotational behavior increased again, leaving no statistical difference between the control and experimental group 4 weeks postremoval. In vivo microdialysis experiments were performed in 6 animals which had received DA-EVA rods. DA-releasing EVA rods: 3 acutely and 3 after 7 days. Twenty min after the implantation of a 20% DA-releasing EVA polymer rod, detectable levels of DA were recovered. The DA levels remained elevated throughout the next 180 min. Significant extracellular striatal DA was present in the lesioned striatum 7 days postimplantation of DA/EVA:Ac rods. Suspended release of dopamine from a polymeric matrix placed within a semipermeable receptive alleviates experimental parkinsonism in rats. This technique offers the advantage of direct target access while preventing subsequent damage during placement or retrieval of loaded polymer matrices.


Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the synthesis of catecholamines and has been implicated in several human neuropsychiatric disorders. In order to produce defined clonal transplantable cells, the recombinant vector pBDLMP10 containing cDNA for form 2 of human tyrosine hydroxylase (HHTH-2) was used to transfer human TH activity to NIH-3T3 cells. Transfected cells were examined for L-DOPA-resistant transferred 3T3 fibroblasts and enzyme kinetics of THTH-2 were characterized. Northern blot analysis using a 1.9 kb probe derived from rat TH identified mRNA species of 5.3 and 5.5 kb in NIH-3T3 cells transfected with THTH-2. The size of endogenous rat adrenal mRNA was approximately the same size as THTH-2, therefore, allowing that both the transfected and endogenous mRNA species co-exist. Analysis using antiserum against bovine TH revealed an immunoreactive band with an apparent molecular weight of 62 kD in these same cells. TH-like immunoreactivity was detected in the transfected 3T3 cells. THTH-2 was characterized using a modification of the assay described by Ngoy et al. (1994). The Vmax of the TH activity of 2.2 µM was 210 times that of endogenous rat adrenal TH activity. Tyrosine hydroxylase activity was not detectable in NIH-3T3 cells transfected with pBDLMP10. When HTH2-3T3 cells were grown in media supplemented with L-tyrosine (1 mM) and B64 (1 mM), the concentration of L-DOPA in the incubation media was 16 µM after 24 hours. L-DOPA production required B64 and was blocked by addition of a competitive inhibitor of TH, 3-iodothyronine.

These results demonstrate that the human TH gene can be transferred to eukaryotic cell lines and that active tyrosine hydroxylase is produced. The efficacy of utilizing these transplantable cells producing micromolar concentrations of L-DOPA in the treatment of dopamine-deficiency states is being investigated.
534.11

Kiesel-ram infected PC12 cells survive better than naive PC12 cells after intracerebral transplantation in adult rat. O. Okada* and V. W. Wrightman, Lab of Neurobiology, WINSL, 711, Berwyn, IL 60516.

The rat pheochromocytoma cell line PC12 can be induced to express neuronal features by Kiesel-ram virus infection (Kosaka et al., Nature 318:733). The K-ram infected PC12 cells have extensive neurites and contain choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH), indicating both cholinergic and adrenergic features. K-ram infected PC12 cells were injected into the caudate-putamen of adult Sprague-Dawley rats and the same number of naive PC12 cells were transplanted into the opposite striatum as a control. At two weeks after transplantation, there was usually a big hemorrhagic lesion associated with the naive PC12 cells. In contrast, the K-ram infected PC12 cells appeared as a mass of cells with less inflammatory reaction from the host tissue. By four weeks, grafted naive PC12 cells had disappeared from the host brain in most cases while some K-ram infected PC12 cells consistently remained in all specimens. These surviving cells were positive for both ChAT and TH and had cellular processes.

K-ram infected PC12 cells survive better than naive PC12 cells following transplantation in adult rats and may serve as a continuous source for both cholinergic and adrenergic transmitters without the need of exogenous, nerve growth factor stimulation.

534.13


Rat PC12 cells infected with a retroviral vector containing cDNA encoding mouse beta-NF, become amnestic and extend neuritic processes in vitro in a manner similar to PC12 cells treated with exogenous NF. The present studies were undertaken to determine whether these cells contribute to express neuronal characteristics following implantation into the striatum of adult mice in which striatal dopaminergic fibers were lesioned with 1-methyl-4-(2-methylphenyl)-1,2,5,6-tetrahydropyridine.

Twenty days post-implantation, many grafted cells survived as assessed by immunoreactivity for NF and TH, and had cellular processes. Although most of the genetically modified cells were immunoreactive for TH in vivo prior to implantation, at twenty days-post-implantation many of the NGFR-positive grafted cells had disappeared from the striatum while a small number of TH-immunoreactive cells were not immunoreactive for NF, suggesting a change of phenotype.

534.14


Unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (SN) selectively destroy dopaminergic neurons and induce rotational behavior in rats. Fetal grafts of SN into the caudate nucleus reverse the behavioral deficit associated with this lesion. Presently, we are using this lesioned-induced behavior model and neural grafting to evaluate changes in gene expression within the nigrostriatal system. In situ hybridization has been used to show the presence of tyrosine hydroxylase (TH) mRNA within grafted dopamine (DA) cells, and preliminary data suggest that the presence of a fetal SN graft in the intact striatum may down-regulate TH gene expression in the ipsilateral host SN. In addition, we and others have shown that disappearance of a specific TH hybridization signal over the lesioned SN coincides with elevated levels of preproenkephalin mRNA in the ipsilateral striatum. This model is being used to study how the reintroduction of DA via neural grafting affects the expression of a variety of genes. For example, does the striatum respond to DA denervation by increasing trophic factor gene expression? One such candidate is nerve growth factor receptor mRNA, which is transiently expressed in the striatum during development. Further studies will be aimed at quantifying measures of gene expression in the nigrostriatal system.

Support: N.I.H. MSTP T32 GM07356 (L.K.), the Pew Foundation (J.S.), and a Mallinckrodt Scholar Award (G.H.).
TROPHIC FACTORS AND THE AGONIST-STIMULATED ACCUMULATION OF INOSITOL PHOSPHATES IN HIPPOCAMPAL SLICES. M.C. Bonner1, P. Tandon2, and M.A. Tilson3. 1 Curriculum in Toxicology, UNC-Chapel Hill, NC 27599 and 2 LMIN, NIH/NIH, Research Triangle Park, NC 27709.

This study examined the influence of electric fields on recovery of physiological and histological characteristics of rat muscle following denervation.

An electric field was applied to the left sciatic nerve transsected and reanastomosed using a silicone cuff with electrodes attached to either end. The electrodes were connected by flexible wire to the control unit which was implanted subcutaneously. At 2, 3, and 4 weeks later twitch tension was measured and histological and histocinical characteristics were assessed.

This study indicates that electric fields can improve the recovery of the nerve injured.

Supported by NIH-NINDS-NS-01334. Traxon devices supplied by American Biointerface.


We have performed a new approach to the study of the interaction between Schwann cell and neuronal processes. Schwann cell cultures were prepared from the sciatic nerve of 2 day old rat in the presence or absence of NGF. Schwann cell cultures were examined under the microscope and the number of axons growing on Schwann cell processes was counted.

The findings were: Schwann cells synthesize and express nerve growth factor (NGF) and the ability of Schwann cells to stimulate axonal growth is increased by NGF.

Supported by NIH-NINDS-NS-01334. Traxon devices supplied by American Biointerface.


We have observed that denervated muscle developed a new phenotype during recovery. This may be due to an increase in the conversion of slow twitch muscle to fast twitch muscle.

Supported by NIDCD-NS-01334.
Preventing the death of NGF-deprived sympathetic neurons. (Ibid) or maximally inhibit protein synthesis in higher (25-100 ug/ml) than required to rescue sympathetic neurons. Blocking protein synthesis directly with CHX prevents the death of NGF-deprived sympathetic neurons. Prior to an experimental manipulation, a retrogradely transported fluorescent tracer (fluoro-gold) was injected into the target muscle. When combined with silver staining of axons and compared with a control probe, intense hybridization was seen in the early nodose ganglion compared with the hybridization intensity of the dorsal root ganglia, sympathetic ganglia and the lateral motor column in the spinal cord. Surprisingly, high hybridization levels were also seen in the nodose ganglion at later stages. Labeling with different intensities were mainly observed over large differentiated sensory neurons in the nodose ganglion cell bodies. The same sections were then hybridized in-situ with cDNAs coding for gene products of interest. Cells previously identified by fluorescence labeling were perfused with pargyline and relevant spinal cord sections photographed with a computer-aided video analysis system. Contralateral and different-animal same-slide controls provided the basis for semiquantitative comparison of gene expression.

**535.9**

**SERVE GROWTH FACTOR RECEPTOR EXPRESSION IN INTRAMUSCULAR NERVES : INDUCTION BY BOTULINUM TOXIN. K-C. Yee, University of Manitoba, Winnipeg, Manitoba R3E 0L2, Canada.**

Nerve growth factor receptor (NGFR) expression by Schwann cells following axotomy of peripheral nerves, including motor nerves, led to the postulate that it is triggered by loss of axon-Schwann cell contact. In this study, NGF expression in intramuscular nerves was examined following pharmacologic denervation by botulinum toxin. Adult rat muscles and rhomboid muscles were directly injected with botulinum toxin. Thick longitudinal cryostat sections of muscles removed 1 to 28 days later were stained with a combination of intramuscular-NGF receptor antibody, a monoclonal antibody to NGFR (192-ig, courtesy Dr. M. Johnson) and a PAP technique.

In control muscles, NGF staining was generally absent in motor nerves but strongly positive in vascular autonomic and intrafusal sensory nerves. Following botulinum treatment, motor nerves at all time points showed a similar pattern of staining extending to the motor endplates. When combined with silver staining of axons and compared with 5-100 staining of Schwann cells, NGF staining was localized to Schwann cell surfaces of intact motor nerves. These findings suggest that factors other than loss of axon-Schwann cell contact may trigger NGF expression by Schwann cells of motor nerves. Potential factors may be derived from the denervation of muscle or induction of motor neuron sprouting caused by botulinum toxin.

**535.11**

**INHIBITION OF PROTEIN SYNTHESIS PREVENTS CELL DEATH IN SENSORY NEURONS DEPRIVED OF NEUROTROPHIC FACTORS. S. A. Green, B. S. Xu, M. F. Gage, Dept. of Anatomy, St. Georges Hosp. Med. Sch., London, UK, andone.**

During embryonic development in vivo, peripheral neurons become dependent on a neurotrophic factor, such as NGF, for survival. Recent studies have demonstrated that inhibiting protein synthesis prevents NGF-dependent cell death of sympathetic neuroblasts in culture. We have examined whether the effects of NGF on sympathetic neurons can be extended to an active cell death program. To determine whether the trophic interactions of other neuronal populations involve similar mechanisms, we have examined the effects of inhibiting protein synthesis on the survival of spinal cord neurons defined by their innervation of a single target muscle. We can thus investigate the effect of various manipulations of the target on gene expression in the innervating motoneuron. To determine whether the NGF-deprived sympathetic neurons. Recent studies have suggested a possible role for NGF in the central nervous system. It is far from clear, however, whether a deficiency of NGF is responsible for impairments in learning and memory. We report here that continuous intracerebroventricular infusion of a specific anti-NGF over a period of four weeks produces signs of impaired memory in the Morris's water-maze task during the anti-NGF infusion period. The swimming and goal latency in the control rats rapidly shortened day by day, compared to that in the anti-NGF treated rats. The degree of habituation to the new environment in the anti-NGF treated rats was significantly lower than that of the control rats. The step-through latency in the anti-NGF treated rats was significantly shorter than that of the control rats. The experiments reported here have developed a model in the rat to study physiological changes due to a deficiency of NGF in the adult central nervous system.

**535.12**

**NEURAL EXCITABILITY IS MAINTAINED IN ADULT RAT SENSORY NEURONS CULTURED IN SERUM- AND EXOGENOUS GROWTH FACTOR-FREE MEDIA. Luis G. Aguayo, Emilio E. Weight and Geoffrey White*, Sect. Electrophysiology, LPPS, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md 20852.**

It has been suggested that deprivation of nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) may not be essential for the survival of adult peripheral neurons. In order to investigate neural excitability and its regulation by growth factors, we have established a method of culture and recording from individual sensory neurons dissociated from adult rat dorsal root ganglia. When cultured in serum-free media, neurons (15-30 μm) survived for over 3 weeks and extended long processes. Whole-cell recordings revealed that the neurons had resting membrane potentials negative to -50 mV. Application of depolarizing outward current steps elicited overshooting action potentials (duration < 2.5 ms) in most of the cells examined. The amplitude of the action potential was sensitive to local application of TTX (1μM) but not Cd++ (500 μM). Replacing intracellular K+ with Cs+ greatly prolonged the duration of the action potential. Voltage-clamp recordings demonstrated the presence of inward and outward currents. From a holding potential of -100 mV, depolarizing steps activated a fast inward Na+ current that was sensitive to TTX, and a slow inward current that was blocked by BAPTA. From these observations, we have established a methodology for maintaining and recording from sensory neurons in vitro.
MONOCONAL ANTIBODIES TO THE CELL SURFACE AND A SOLUBLE FORM OF HUMAN NERVE GROWTH FACTOR RECEPTOR. M. Chugani, C. Chugani, Y. Chang, P. Meriläinen, F.E. Miami, and D.D. Neurology Research Institute, Div. of Nerv. Dis. and Dept. of Neurosurg., Miami, FL 33104 and Dept. of Pathol., Univ. of Miami, Miami, FL 33101.

Monoclonal antibodies (mAbs) have been produced to a soluble, truncated form of the human nerve growth factor receptor (NGF-R). NGF-R was obtained from the conditioned medium (CM) of E9 calf embryo fibroblasts (CEF) collected in that experiment was the human NGF receptor (NGF-R) on the cell surface. (Chao, M.V. et al. Science 232:518, 1986). The soluble receptor, in CEF CM binds [125I]-NGF, and after chemical crosslinking, a 50,000 Mr protein is immunoprecipitated by a mAb (ME20.4) to the cell surface. In CEF CM, the cell surface NGF-R is a pre-protein, partially purified by immunofinity chromatography (~105 fold) and used to induce hybridomas. Hybridomas were screened by radiometric immunosorbant assay and immunopurification of solubilized cell surface receptor covalently crosslinked to [125I]-NGF after purification and deletion of the I-gal, subfamily. All mAbs that bind to the NGF-R and NGF-Rf. Two mAbs immunoblot a single protein corresponding to the NGF-R after resolution of E9 membrane proteins on non-reducing SDS-polyacrylamide gels. All mAbs are specific for human NGF-R and do not cross-react with receptor from other species. Antibody competition studies indicate that these antibodies bind to a single epitope on the NGF-R, and one mAb bind to a distinct protein epitope. Antibodies to different epitopes have been used to develop a two site radiouunosorbant assay to quantitate NGF-R in culture medium and human samples.


This study used an affinity purified monoclinal antibody against NGF receptors (IgG 192, kindly provided by Dr. E Johnson, University of Rochester Medical Center) to study NGF-R expression on astrocites and neurons in adult human brain tissue. Reactions for NGF-R immunoreactivity were observed in subependymal, subependymal, and ependymal astrocytes and neurons in the human brain. NGF-R immunoreactivity was increased on the lesion side M.S.-V.D.B. complex for both the bFGF treated and NGF-R deficient rats, suggesting that NGF-R immunoreactivity increases in astrocytes and neurons in response to NGF-R deficiency. These data indicate that the NGF-R is upregulated in astrocytes in response to NGF-R deficiency.
**536.3**

**NEUROTROPHIC ACTIONS OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS ON SPINAL CORD NEURONS.** SR Whittemore, IM Sweatt, LM Sanger, EB Breese, FF Strawson. Dept. of Neurosurgery, Physiology & Biophysics, & Pharmacology, Univ. of Miami Sch Med, Miami, FL 33136.

Under conditions which minimize substrate effects (7%CO₂, 10%CH₃OH, 10%CH₃COOH) acidic basic FGF on E12.5 spinal cord choline acetyltransferase (CHAT), glutamic acid decarboxylase (GAD), and aspartate aminotransferase (AST) activities were determined. cFGF had no effect on CHAT, GAD, or AST (0.1-100 ng/ml) in cultures grown with or without FGF. cFGF did increase protein synthesis in cultured neurons. cFGF increased CHAT activity (EC₅₀ 50ng/ml) by 40% in the absence or presence of FGF, and slightly increased GAD and AST.

Western blotting could not detect aFGF or bFGF, nor could 300, 600 or 1000 ng/ml FGF/FGF protein. The remaining bFGF was intracellular. Inhibition of proteoglycan synthesis decreased levels of all FGF forms.

Results suggest that multiple neuronal enzyme activities by a common mechanism of action which may be mediated through bFGF with astrocyte ECM.

**536.4**

**EPIDERMAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR ACT AS OVERLAPPING POPULATIONS OF NEUROTROPHIC NEURONS.** IL Kambham, HC Raymond, RS Morrison, K.P. Cavagnero, R. A. Bradshaw, E.M. Leslie, U.C. Irvine, Irvine, CA 92717 and Albert Einstein College of Medicine, Bronx, NY. 10467.

Recent studies have demonstrated that epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) serve as trophic factors for CNS neurons. In the present study, we demonstrate that neurotrophic factors respond to both neurotrophic agents. EGF and bFGF. However, EGF appears to affect a larger subset of neurons. Dissociated neuronal cultures were prepared from newborn rat brains and plated in DME/F12 medium supplemented with 10% fetal calf serum on polylysine-coated dishes. One day after plating, cultures were switched to a chemically defined medium and appropriate growth factors were added. Dose response studies indicated a maximal effect on process outgrowth and cell survival with 10 ng/ml EGF and 30 ng/ml bFGF. bFGF had an effect on approximately 15% more neurons than EGF. The combination of EGF- and bFGF-induced only slightly higher effects on process outgrowth and cell survival, than with either alone. Neuronal survival was maintained in cultures grown in the presence of EGF and then switched to bFGF, while EGF had a lesser effect on cultures grown in the presence of EGF and bFGF had a differential morphological effects on cortical neurons. bFGF induced a greater number of processes per neuron than did EGF at maximal stimulatory concentrations. These data indicate that bFGF supports the survival and process outgrowth of an overlapping population of neurons with bFGF affecting a greater percentage of plated neurons. This work was supported by NIH NS 19179.

**536.5**

**UNASSIGNED BASAL FOREBRAIN CHOLINERGIC NEURONES ARE NOT DEPENDENT UPON LIMITING AMOUNTS OF TARGET-DERIVED FACTORS FOR SURVIVAL IN THE ADULT.** N.P. Galletly, C.N. Svendsen, O. Isacson and M.V. Sofroniew. Department of Anatomy, College of Medicine, Houston, Texas 77030.

Neonatal rat superior cervical ganglion (SCG) neurons in culture can express cholinergic properties under the influence of several factors including choline acetyltransferase inducing activity from heart conditioned medium (HCM), ciliary neurotrophic factor from rat 3T3 cells, gastrin releasing peptide (GRP) and membrane associated neurotransmitter inducing substance (MANS) from rat cardiac tissue. To determine if these factors are related, we have compared their biological activity in several assays and used antisera generated against CDF in immunoprecipitation experiments. As previously reported, CDF, ciliary neurotrophic factor (CNTF), MANS and CDF all show cholinergic inducing activity when added to SCG cultures. In addition, both crude and partially purified preparations of CNTF and MANS promote survival of EGF chick ciliary neurons in culture. In contrast, CDF does not promote ciliary neuron survival. Polyclonal antibodies generated against CDF and CNTF (Pakuda, 1986) immunoprecipitated a 45Kb protein from 1125 labelled HCM from experiments with labelled CNTF and MANS fractions, no specific protein bands are precipitated. This indicates that CNTF and MANS, but not CDF, induce cholinergic inducing activity or the ciliary neurotrophic activity or the ciliary neurotrophic activity from CNTF and MANS can be precipitated by CDF antisera at concentrations that will immunoprecipitate all cholinergic inducing activity from HCM. Thus, CDF is biologically and immunologically distinct from CNTF and MANS. Supported NIH, McKnight Foundation and the ARA.
THE ROLE OF GANGLIOSIDE IN MEDIATING NEUROTROPHIC FACTOR RECEPTOR (NGF-R) GENE EXPRESSION IN RAT CNS. Soo Young Koh and Gerald A. Higgins. University of Rochester Medical Center, Rochester, NY 14623.

During CNS development, there is a peak of NGF synthesis in the projection areas of basal forebrain neurons. This developmental change in NGF production is paralleled by an increase in the perikaryal size of NGF-responsive basal forebrain neurons during the third postnatal week, suggesting a possible role for endogenous NGF in the mediation of basal forebrain neuronal hypertrophy. In order to determine whether an induction of NGF-R gene expression occurs during the postnatal period, we have used mRNA in situ hybridization to study the regulation of NGF-R mRNA within individual neurons of rat basal forebrain. 

35S-labeled antisense NGF-R RNA probe was generated by in vitro transcription of a 1.7-kb NGF-R cDNA, size-reduced by alkaline hydrolysis, and hybridized under stringent conditions to pretested brain sections at postnatal day (P5, P15, P16, P22, and P30). Increases in NGF-R mRNA content can be observed at P5, is more marked at P10, and reaches almost four times the level of mature neurons by P15. The peak of expression is followed by an abrupt fall in the message level at P22, and the further decrease at P30. Induction of NGF-R mRNA may thus render the cells more responsive to NGF and augment a cascade of NGF-mediated trophic actions to result in neuronal hypertrophy. In developing cerebellum, the highest levels of message are detected in Purkinje cells and the external granular layer, suggesting that Purkinje cells and immature granule cells may be sites of NGF-R biosynthesis.


Tyrosine hydroxylase (TH), the first and rate-limiting enzyme for catecholamine biosynthesis, is subject to regulation by cell contact and NGF. It has been shown that TH mRNA levels were induced in PC12 cells grown at high densities whereas NGF treatment decreased TH mRNA levels. Regulation of TH alone, however, does not specify the particular catecholaminergic phenotype. Therefore, we examined dopamine β-hydroxylase (DBH) mRNA levels in PC12 cells grown at various cell densities, and/or for various times with or without NGF (50 ng/ml). Quantitation of northern blot analyses revealed that at 10-20 fold higher densities there was up to an 18-fold increase in TH mRNA levels, but DBH mRNA levels decreased slightly (~50%). There was a 5-fold increase in cellular dopamine content but virtually no change in norepinephrine levels as measured by HPLC-EC. NGF treatment for up to 8 days led to a decrease in TH mRNA levels (~40%) while DBH mRNA levels rapidly decreased by as much as 85%. NGF treated PC12 cells (4 days) regressed to high density still demonstrated an induction of TH mRNA, however, DBH mRNA levels decreased to undetectable levels. Thus, increased cell contact differentially regulated TH and DBH mRNA levels suggesting that cell contact promotes a more dopaminergic phenotype whereas NGF treatment of PC12 cells decreases both TH and DBH mRNA levels.


Exogenous gangliosides potentiate the action of several neurotrophic factors including Nerve Growth Factor (NGF) and factors in glial conditioned medium (GCM). Previoiusly, we have shown that ganglioside GM1 potentiated NGF-mediated and GCM-mediated neuritogenesis of chick embryonic sensory ganglia (EG) during development. No GM1 potentiation was observed during the peak of NGF-mediated development, embryonic day (ED) 8 to 10; on other days, treatment with GM1 and/or NGF enhanced growth. In contrast, cells grown at various cell densities, and/or for various times with NGF (50 ng/ml). Quantitative northern or slot blot analyses revealed that at 10-20 fold higher densities there was up to an 18-fold increase in TH mRNA levels, but DBH mRNA levels decreased slightly (~50%). There was a 5-fold increase in cellular dopamine content but virtually no change in norepinephrine levels as measured by HPLC-EC. NGF treatment for up to 8 days led to a decrease in TH mRNA levels (~40%) while DBH mRNA levels rapidly decreased by as much as 85%. NGF treated PC12 cells (4 days) regressed to high density still demonstrated an induction of TH mRNA, however, DBH mRNA levels decreased to undetectable levels. Thus, increased cell contact differentially regulated TH and DBH mRNA levels suggesting that cell contact promotes a more dopaminergic phenotype whereas NGF treatment of PC12 cells decreases both TH and DBH mRNA levels.


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We have previously extracted a 5-HT neurotrophic signal from the 5-DHT lesioned hippocampus (Zhou et al., J. Neurosci. Res., 17:235, 1987). Current study shows that a new 5-HT neurotrophic factor was monitored in a DA-5-HT striatal model.

A 5,7-DHT lesioned hippocampus (5,7-DHT, 50ug/10ul ascorbic saline, two bilateral injections) lesion in the nigra specifically removed the dopamine (DA) neurons in high limbic and DA-territorial fiber tracts. In response to the DA-denervation, with immunocytochemical staining, we found that the 5-HT fiber grows vigorously in the previously sparsely innervated striatum. The 5-HT fibers normally innervate the striatum sparsely, especially densely with sharp delineation (in control side), became dense across both areas with no appreciable delineation (in lesion side). The increase of 5-HT fibers was more prominent in postlesion than in anterior side.

HPLC measurement supported this view. Significant increase in 5-HT and 5-HIAA level was evident in the posterior striatum when the decrease of DA level exceeded 90% in nigra and striatum. In addition, we found that when the decrease of DA level in substantia nigra and striatum was less than 90%, the 5-HT and 5-HIAA levels were not significantly changed in the striatum or pallidus. The induction of 5-HT sprouting requires a >90% decrease of DA content.

The induction of 5-HT sprouting requires a >90% decrease of DA content.
c-fos GENE EXPRESSION FOLLOWING CORTICAL INJURY AND INTRACEREBRAL INJECTIONS OF NGF AND EGF. F.B. Sharp, M.F. Gonzalez, and S.M. Sagar, Depts of Neurology and Physiology, UCSF and VA Med Ctr, SF, CA 94121.

Small lesions of rat motor cortex induces Fos-like immunoreactivity and c-fos mRNA expression in neurons throughout the ipsilateral neocortex. To explore the possible involvement of growth factors in this injury induction of the c-fos proto-oncogene, NGF (3ug) was injected into rat motor cortex. This induced Fos, the protein product of the c-fos gene, in neurons throughout neocortex 24 hours after the injection. Saline control injections had little effect. EGF (3ug) was also injected into rat motor cortex and hippocampus. This growth factor induced Fos in presumptive oligodendroglia in corpus callosum, in endothelial cells in cortex and hippocampus, and perhaps in astroglia around the local site of injection.

These data suggest that trophic factors induce Fos in an anatomic pattern that depends on the nature of the trophic factor and receptors on the target cells. The injury induction of Fos throughout neocortex is consistent with the local release of NGF and EGF at the cortical injury site. Though the cellular function of Fos is still unknown, these results show that trophic factors can produce diffuse, prolonged Fos-mediated metabolic changes in neurons throughout neocortex.


The magnitude of axotomy-induced neuronal loss in adult female rats could be attenuated by the administration of testosterone or progesterone (Yu, W.H.A., J. Neurosci.; Brain Res., in press). The present study investigates whether progesterone (P) acts as a precursor for testosterone (T) or P is effective by itself in reducing neuronal loss. Three-week-old male and female rats were subjected to unilateral transection of the hypoglossal and facial nerves, and injected i.c.v. with 0.5 or 2.0 mg P in 0.1 ml sesame oil twice weekly for the first 4 postaxotomy wks and once weekly for 6 additional wks. Controls received oil vehicle injection. Neuronal numbers in the hypoglossal and facial motor nuclei were counted 15-11 wks after axotomy in serial paraffin sections stained with cresyl violet. Results indicate that P treatment with either dose significantly reduced neuronal loss in the hypoglossal nucleus in females, but the same treatment did not alter the magnitude of neuronal loss in males. Since the administration of T to prepubertal male rats adversely caused an increase in neuronal loss similar to the condition seen after orchidectomy (unpublished data), the presence of response to P treatment in females but not in males suggests that P promotes neuronal survival not by the enzymatic conversion to T. However, a gender difference in the amount and/or the time course of the acquisition of the conversion enzyme may exist.
537.3

OLFATORY NERVE INGROWTH INDUCES TYROSINE HYDROXYLASE EXPRESSION IN RAT FOREBRAIN NEURONS. K. M. Guthrie, C. M. Gall and M. Leon. Deps. of Psychiatry, and Anatomy & Neurobiology, University of California, Irvine CA 92717

Functional olfactory nerve input is required for the normal expression of tyrosine hydroxylase (TH) by dopaminergic neurons in the glomerular layer of the rodent main olfactory bulb. To determine whether olfactory nerve input exerts a similar influence on cells in other brain regions, we used the olfactory nerve to innervate the rat forebrain. To accomplish this, we performed unilateral bullectomies in rat pups on postnatal day 5-7 and examined the brains 2-6 months later, after the regenerating nerve fibers had reached the brain. Tissue was stained for TH-, dopamine B-hydroxylase (DBH-), and olfactory marker protein (OMP)-immunoreactivity. In addition, in situ hybridization histochemistry using a 35S-labeled riboprobe was used to examine the forebrain for TH mRNA.

We observed expression of TH-immunoreactivity and mRNA in neurons located in areas of the forebrain which received ingrowing olfactory nerve fibers, particularly along the rostral extension of the subependymal layer. Many of these neurons resembled the periglomerular cells of the olfactory bulb. No cell staining for DBH- or OMP-immunoreactivity was observed in these areas, suggesting the possible dopaminergic phenotype of these neurons. Since it takes several weeks for the regenerating nerve fibers to reach the forebrain, this is the first demonstration of induction of novel expression in the mature brain.

537.5

EXPRESSION OF SODIUM CHANNELS ON HYPOMYELINATED CENTRAL AXONS. J. L. Neocleous, P. K. Marcrom and N. H. Jallilian Tahmasi. Developmental Neurosurgery Laboratory, Dept. of Neurology, 'Biochemistry, and Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas, 77030.

A deletion at the shiverer locus (chr. 18) specifically eliminates myelin basic protein gene expression in mouse oligodendrocytes, resulting in loss of myelin and in the absence of compact myelin formation throughout the central nervous system. One unexpected neurochemical phenotype of the MBP-/- hypomyelinated brain is the preservation of motor strength, indicating intact impulse conduction in large-caliber corticospinal axons. To identify the underlying excitability mechanisms, we performed autographic recordings of frozen 20um brain sections following specific external membrane binding with the sodium channel alpha-subunit ligand [3H]-STX incubated at 4C in 20mM Tris-HCl buffer, pH 7.4, containing 140mM N-methylglycine, 5.4 mM KCl, 2.8 mM CaCl2, and 1.3 mM MgSO4. Densitometric comparisons of toxin binding patterns revealed striking increases over hypomyelinated shi/shi fiber tracts relative to control +/- pathways. Binding was particularly elevated in transcallosal cortical association fibers, optic nerves, fimbriae, and corticofugal tracts. Scatchard analysis performed on homogenized shi/shi and +/- optic nerves revealed specific binding to a single site, and a 4-fold increase in the number of shi/shi STX sites, with no alteration in toxin affinity. Since few sodium channels normally exist in unmyelinated axon internodes, these data show that central neurons possess the developmental potential to modify axonal excitability in response to altered axo-axonial glial interactions by increasing sodium channel expression.

Supported by NIH 11355, March of Dimes, and Pew Scholars Award (JLN).

537.6

ACTIVITY-DEPENDENT REGULATION OF MUSCLE N-CADHERIN. C. C. Hahn* and J. Covault. Department of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Denervation of muscle leads to the redistribution of factors which promote its reinnervation. Nerve activity suppresses the expression of these factors. The adhesion molecule NCAM is thought to be such a factor, mediated in its expression in muscle fibers is down regulated by nerve-evoked activity. We have found that expression of the adhesion molecule N-cadherin in similarly regulated muscle fibers is particularly abundant on the surface of embryonic chick myotubes but is markedly reduced within 3-4 days of their innervation. A major effect of the decrease in innervation on the undetectable intravascular fibers  but was induced by surgical denervation. The effects of denervation could be mimicked by TTX blockade of nerve conduction indicating that N-cadherin expression is normally inhibited by nerve-evoked electrical activity.

Activity-dependent regulation of N-cadherin and NCAM can be reproduced, at least in part, in myotube cultures. TTX blockade of spontaneous activity for 2 days caused a 20-25% increase in N-cadherin and a 30-40% increase in NCAM. The effect of TTX was reversed by the Ca channel antagonist ryanodine suggesting increased intracellular Ca secondary peaks in the interval histogram were in the "tonic" range, so that chronic TTX treatment had resulted in an overall shift towards very high frequency discharges, but separated by relatively long silent periods in comparison with normal firing patterns. Such a change could have resulted from a general increase in membrane polarization levels, whereby spikes become more difficult to elicit but, once triggered, tend to occur repetitively in rapid succession. Up-regulation of GABA receptors in the course of the prolonged period of blocked synaptic inhibition could thus account for the observed pathophysiology.

537.7


We examined the electrical properties of cat spinal motoneurons (MNs) supplying muscles paralyzed by type A botulinum toxin (B'TX). A dose of about 25 ng of B'TX (1.0-1.5 ml saline) was injected into the medial gastrocnemius (MG) muscle. After 2-3 weeks, the animals were prepared for intracellular recording from MG MNs. Animals were divided into two groups based on their response to nerve stimulation. Twitch tensions of one group (low force) were < 5.0 gms (mean 4.4 gms); the other group (high force) had twitch tensions > 20 gms (mean 15.7 gms). Electrical properties of MNs from low force group were characterized by increased after-hyperpolarizations (negative current, < 200 uA) and spike amplitudes (200-250 uA). Spontaneous activity was also absent or very low in low force group (1-2 spikes per 1000 sec) compared to the high force group (several hundred spikes per minute).

The electrical properties of connected MNs were normal while properties of unconnected MNs appeared axotomy-like. One interpretation of these results is that inhibition of transmitter release results in the absence of mechanical damage to the axon. Supported by NIH grants NS24860 and NS54707.

537.8


Both slow and fast fibres have been characterized in the cruralis muscle of the isolated Bufo arenarum; normally, the slow fibres which in this muscle are abundant and easily penetrated by microelectrodes, do not exhibit action potentials, but after denervation of the cruralis, they developed strong action potentials in response to depolarizing current injection. Action potentials occurred in cruralis slow fibres 25 days after the sciatic nerve was transected at hip level. The latency was reduced to 0.25 m when the nerve was cut where it entered the muscle, and to 8.6 by injecting α-bungarotoxin into the muscle at denervation. The developmental of action potentials also showed a seasonal dependence, occurring in summer and not in winter. Cruralis slow fibres survived in organ culture for 5-10 days; slow fibres of denervated muscles changed little in Vm or Rm, but showed a significant decrease in t, reflecting a decreased capacitance of the cells. The effect of α-bungarotoxin on the latency of the response suggested that Ach may play a neurotransmitter role. But slow fibres of denervated muscles cultured in the presence of an Ach agonist (1-5 mM carbachol) developed regenerative responses as usual. Our results suggest that synapse elimination may be an important regulatory factor in the cruralis slow fibres, but that Ach alone does not seem to be the critical factor.
CHARACTERIZATION OF GIP-70, A GUANETHIDINE-INDUCED, NGF-REGULATABLE PROTEIN EXPRESSED IN SYMPATHETIC GANGLIA.

Monctano Co., St. Louis, MO 63198.

Guanethidine is an adrenergic neuron blocking agent. Chronic administration of guanethidine to rats, hamsters and monkeys induces an autoimmune, cell-mediated destruction of sympathetic neurons. Neuronal destruction can be completely prevented by concurrent treatment with nerve growth factor (NGF). We have previously identified a protein, guanethidine-induced protein 70 (or GIP-70), by 2-dimensional gel electrophoresis which is induced in sympathetic ganglia following guanethidine treatment. This protein is not found if rats are treated with guanethidine and NGF concurrently. GIP-70 has been hypothesized to be a candidate antigen involved in this immune response. To further characterize GIP-70, the protein was isolated in pure form from homogenates of sympathetic ganglia from 550 guanethidine treated rats using preparative 2-D gel electrophoresis. The spots corresponding to GIP-70 were excised, eluted from the gel, and digested with trypsin. Four tryptic fragments, separated by HPLC, were sequenced using Edman degradation gas phase sequence analysis. All four peptides exhibited significant homology to the deduced sequence of the αβ-interferon-induced mouse Mx protein. In addition, GIP-70 and Mx have similar molecular weights and isoelectric points. GIP-70 thus appears to be the rat homolog of mouse Mx. Experiments are in progress to determine whether rat Mx protein is involved in the induction of the guanethidine-induced immune response.

A MONOCLONAL ANTIBODY FOR PROTEASE NEXIN 1 (PN-1) IS STRONGLY IMMUNOREACTIVE IN ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS OF THE BLOOD VESSELS OF HUMAN CEREBRUM.

(Spon: S. van den Noort) University of California at Irvine, Irvine, CA 92717.

Protease nexin 1 (PN-1), also called glial derived nexin (GDN), is a 43-kd protein that is secreted by a variety of cultured cells including fibroblasts, smooth muscle cells and astrocytes. Although PN-1 was first purified from conditioned medium of cultured human fibroblasts the presence of relatively large amounts of PN-1 in both human and rat brain has also been demonstrated. A panel of monoclonal antibodies to PN-1 was produced by fusing P3X-myeloma cells with splenocytes from a Balb/c mouse previously immunized with highly purified human PN-1. Supernatants of one of the resulting hybridomas (C1B5), which are found to be specific for PN-1 by both ELISA and western blotting techniques were used for immunoperoxidase staining of paraffin sections of the adult human cerebrum fixed in 2% paraformaldehyde. Highly specific immunoperoxidase reaction of PN-1 is noted in endothelial cells and smooth muscle cells of the blood vessels within the parenchyma as well as leptomeninges of the cerebral cortex. Immunoperoxidase staining using a polyclonal antibody for PN-1 produced almost identical results. It is believed that neurotrophic activity of PN-1 is dependent on its ability to inhibit thrombin, and accumulating evidence suggests that their interplay might be important in the regulation of neurite outgrowth in the brain. Demonstration of PN-1 in endothelial cells and smooth muscle cells of the blood vessels of the brain thus provides an important clue as to the cellular localization as well as to the role of these molecules in development, function and regeneration of the central nervous system. (Supported in part by NIH grants ES05298, GM31609 and CA-09054)

NEUROTGENESIS IN CHROMAFFIN CELLS "IN VITRO:" EFFECTS OF CENTRAL GLIA.

J.A. Colombo, G. Persa, R. Caccini, and G. Gran.
Unidad de Neurobiologia Aplicada (CICM-CONICET), Buenos Aires, Argentina.

Adrenal medulla chromaffin cells have been proposed as a possible cellular replacement therapy in cases of human Parkinson's disease resistant to pharmacological treatment. Their reported ability to shift from an endocrine to a neuronal phenotype may prove to be of interest. Immature (PN 10) or adult rat adrenal medulla were enzymatically and mechanically dissociated. Cells were seeded on polylysine-coated coverslips and cultured in DMEM-horse serum (10%) or DMEM-F 12 media at 37°C and 95% air/CO2 atmosphere. The following groups were analyzed: NGF (2.5-5)100 μg/ml, with or without co-culture on top of a fetal, central glial carpet grown to confluence and physically separated from the chromaffin cells; controls with no glia or NGF were included. Infrequent spontaneous neuritogenesis was observed in control cultures. On the contrary, interaction between NOF and glia tended to support, or induce, a higher incidence of neuritic growth under present culture conditions. This work was supported by the ONC (Argentina).

ALTERED SYNAPSE FORMATION AND REPRESSION IN THE STRIATUM OF ANIMALS PERINATALLY EXPOSED TO MORPHINE.

A. Doria, B. Teocenci, R. La, Corix, A.M. Di Giuliod, and F. Mantegazza
Inst. of Pharmacol. Sciences, School of Pharmacy, via Balzaretti 9 and Istituto Scientifico N.S. Raffaele, via Olgettina 60, Dept. of Medical Pharmacology, School of Medicine, via Vanvitelli 32, Milano.

Rats exposed to morphine during the entire fetal life and postnatally up to the day of sacrifice were utilized in the study. The main affected area is the corpus striatum. Noradrenaline content in this area is higher at postnatal day 12, but later on its levels normalize. Serotonin is also affected by a moderate transient neonatal reduced innervation of the striatum. The dopaminergic innervation is significantly reduced up to day 30 of life. The developmental pattern of striatal net-enkephalin innervation is markedly affected as well, being the peptide levels much higher than controls up to day 60 of life, thus indicating increased synaptogenesis. The pattern of net-enkephalin synaptic repression is similar in normal and morphine-exposed animals. Substance P shows a transient hyperinnervation of the striatum, limited to the first decade of life.

PRODUCTION OF RECOMBINANT EXTRACELLULAR DOMAIN OF HUMAN NERVE GROWTH FACTOR RECEPTOR IN BACULOVIRUS EXPRESSION SYSTEM.

P. Visvaderqualja and A.H. Ross.
Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545

The recombinant extracellular domain (RED) of human nerve growth factor receptor (NGF-R) was expressed in Sf9 (Spodoptera frugiperda) insect cells using a baculovirus expression system. A transfer vector was constructed inserting the cDNA coding for extracellular domain of the NGF-R into pVL1391. Recombinant virus was produced by cotransfecting the DNA of Autographa california multiple nuclear polyhedrosis virus and the transfer vector into Sf9 cells. Recombinant viral plaques were screened for the presence of NGF-R gene and for RED expression. RED-positive virus was plaque purified and used for infection of large scale suspension cultures. The RED was secreted into the cell medium from 48hr to 96hr postinfection and was found to bind [125I]-NGF. RED was purified by ammonium sulfate precipitation, immunoaffinity chromatography and ion exchange chromatography. Approximately, 5-10mg of the RED was produced per liter of suspension culture. The RED has a higher apparent molecular weight by gel exclusion chromatography than predicted suggesting that it exists in solution as a dimer or tetramer. Purification of a second recombinant virus coding for the full length NGF-R is in progress.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 15, 1989
533.1
CGRAFTS OF RETINA AND RETINAL PIGMENT EPITHELIUM TO ADULT RABBIT RETINA. R. Aramant, M. Seiler, A. Bergstrom* and A. R. Adolph.
Eye Research Institute, Boston, MA 02114 and *Dept. of Ophthalmology, University of Lund, 22185 Lund, Sweden.

Co-grafts of retina and retinal pigment epithelium (RPE) are described following transplantation in adult rabbit host retinas. The technique also allows for the survival of the neurones in relation to estimates of the numbers transplanted. The method also provides a means for positive identification of the host and donor cells by means of immunocytochemistry. Transplantation appears to be a promising tool for studying pathogenetic mechanisms operating in genetic retinal degeneration. Supported by NEI grant 50526.

533.2
RETNAL TRANSPLANTATION AS A TOOL FOR THE STUDY OF RETINAL DEGENERATION. L. O. Jiang and M. del Cerro. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14623.

Neuronal transplantation can be used as a tool to study neural degenerative diseases. Retinal transplantation, which provides the opportunity to implant the neural retina into a genetically defined intracellular retinal degeneration in rd/rd mice. The size of the rd gene action remains underdetermined, photoreceptor cell degeneration may be caused by intrinsic cellular factors with secondary changes in the intracellular environment. We attempt to study both possibilities (i.e., >rd/rd and >+/+). C57Bl/6j mice (rd+/+ and AKR/1 mice (+/+) were used. The mice were housed in a standard laboratory condition (12 h:12 h dark/light cycle) and the hosts were perfusion fixed at 6 weeks after transplantation. The samples were examined by routine histological methods and ultrastructural analysis.

533.3
SELECTIVITY OF CONNECTIONS MADE BY RETINAL PATHWAYS OF THE HOST VISUAL SYSTEM. In order to explore the functional potential of retinal connections in host visual nuclei, we have examined the functional potential of retinal transplants. Our data show that by choosing judiciously the donor-host combination it is possible to achieve positive identification of the host and donor cells by means of immunocytochemical or biochemical markers. Supported by NEI grant 50526.

533.4

Successful retinal transplantation requires determination of functional circuitry between neuronal elements within the graft and between graft and host. In the present study, we performed ultrastructural analysis of embryonic retina (E15, E15) transplanted in the visual cortex of neonatal rats. Five weeks after transplantation, the latest time point observed in these studies, the host reaction was characterized by a few macrophages surrounding the transplants. This mild reaction is not associated with disorganization. After 8 weeks, grafts of both types appeared to be considerably smaller with little indication of degenerative processes. Disrupted RPE cells are not in contact with Bruch's membrane and the choroid might lose their influence on retinal cells. This possibility is being further investigated.
Transplantation of developing rodent retina into the retina of adult hosts has already been achieved successfully by us and others. However, the potential of developing primates in retinal tissue has not been established yet. We wanted to test the feasibility of this form of interspecies transplantation since it may offer new opportunities in study host-donor cell interactions as well as provide information concerning the structure and function of donor tissue.

Donors were monkey (5.6-6.9 mm in length) and actually overgrew the confines of the host retina, often expanding into the vitreal cavity. Distinct laminin-positive immunoreactivity was closely associated with the implanted cells, which retained their antigenic phenotype, and human growth hormone (hGH) genes. These cell clones were subsequently isolated, grown to confluency and then implanted into the cortex of adult Wistar rats with a Hamilton syringe. After 3-4 weeks the fibroblasts were examined by immunocytochemical methods with antibodies to hGH and fibronectin. An image analysis was used to compute graft volumes in the brain. First and fourth passage cells were suspended in saline (1 x 10^6 cells/ml) and injected bilaterally into the caudate. Rats were sacrificed at 3 and 8 weeks postoperatively. After varying survival periods up to several weeks, host tissue is fixed and histochemically processed for hGH reactivity. In initial experiments we have found dense cytoplasmic reaction product in cells of varying morphology in the areas of grafts. hGH-positive cells include round, pleomorphic, and pyramidal-like shapes of up to 20 µm in length. Small glia-like cells are also seen near blood vessels and pla.

We have examined the feasibility of a non-retroviral system to genetically modify fibroblasts for the delivery of a gene product to the central nervous system. Primary fibroblasts from Wistar rats were transfected by calcium phosphate precipitation with the plasmid pDNA3. Following a lengthy survival period, the fibroblasts were examined by indirect immunofluorescence for examination of cell survival and the effect of these cells on the host brain tissue. Implanted A7 cells were identified by the presence of bisbenzimide labeling as well as a monoclonal antibody against large T antigen which brightly stains the nuclei of the implanted cells. The grafted cells did not produce neoplastic growth and only some of them survived. They did not induce evident immunological reactions within the host brain as measured by the presence of OX-42, W0/13, W0/12 and OX-2 cell infiltrations. Distinct laminin-positive immunoreactivity was closely associated with the implanted cells, which retained their antigenic phenotype, including the surface expression of CAM. Animals examined after 2-8 weeks of survival and their brains were examined using H & E staining as well as indirect immunofluorescence for examination of cell survival. We wanted to test the feasibility of this form of interspecies transplantation since it may offer new opportunities in study host-donor cell interactions as well as provide information concerning the structure and function of donor tissue. Donors were monkey (5.6-6.9 mm in length) and actually overgrew the confines of the host retina, often expanding into the vitreal cavity. Distinct laminin-positive immunoreactivity was closely associated with the implanted cells, which retained their antigenic phenotype, and human growth hormone (hGH) genes. These cell clones were subsequently isolated, grown to confluency and then implanted into the cortex of adult Wistar rats with a Hamilton syringe. After 3-4 weeks the fibroblasts were examined by immunocytochemical methods with antibodies to hGH and fibronectin. Characteristic patterns of hGH immunostaining in the cytoplasm indicated that the fibroblasts had survived and expressed the reporter gene product. These experiments illustrate the potential of this system as a model to study gene replacement therapy in the CNS.
TIMING AND PATTERNS OF ASTROCYTE MIGRATION FROM XENOGRAFTS OF CORTEX AND CORPUS CALLOSUM.

H.F. Zhou, L. Hatton and H.S. U.* Division of Neurosurgery, UC San Diego and Veterans Administration Hospital, La Jolla, CA 92039

The migration of neonatal rat cortical astrocytes transplanted into the brains of adult hosts has previously been transplanted. However, the effects of different sources of donor astrocytes, and of developmental stage of the recipient on this migration is unknown. Therefore, we grafted Fast Blue labelled astrocytes from neonatal rat cerebral cortex, hippocampus and hypothalamus into neonatal and adult brains by injection with a Hamilton syringe. Dorsal or basal forebrain were targeted in the neonatal hosts, while cortex, hippocampus and hypothalamus were targeted in adult hosts. Transplanted astrocytes derived from the neonatal cerebral cortex migrated extensively throughout the adult brain, using the ventricular walls, glial limitans, vasculature and fiber bundles to guide their movements. However, hypotalamic astrocytes homografted into the adult hypothalampus migrated only within the hypothalampus and basal glial limitans. Likewise, homografted hippocampal astrocytes migrated throughout the adult hippocampus and ventral cerebral cortex only. In both cases, migration routes appeared to be random or unguided. In neonatal hosts, some unguided migration was noted in grafts in all areas. Most migration occurred along the vasculature of the developing brain, and was less extensive than in the adult hosts. These results suggest that the migration patterns of grafted astrocytes are dependent on source organs, and that the scaffolding that defines migration routes in the adult brain are not yet well defined in the neonate.

IMPLANTATION OF IMMATURE ASTROCYTES INTO THE CONTUSED SPINAL CORD: CHRONIC EFFECTS ON FUNCTIONAL DEFICIT AND HISTOPATHOLOGY.


As a previous step to study the influence of enriched populations of glial cells on the survival and plasticity of transplanted neurons, we decided to study wether a population of neonatal oligodendrocytes (OL) was able to survive and develop into mature cells after being implanted into a focally demyelinated spinal cord. Fourteen to 21-day-old Wistar-Lewis rats were ether anesthetized, decapitated and their spinal cord were disassembled by an enzymatic-mechanical procedure. OL were obtained from 389/235, 88/1547 and Severe Ochoa-Ferrer Foundation award to JJLL). OL survived and produce myelin in the host's demyelinated spinal cord induced by an injection of 1/5% lysolecithin one week before. At intervals of one to eight weeks, animals were reanesthetized and intraspinally perfused with appropriate fixatives. The spinal cords were removed and the lesion morphologically analyzed by LM and EM techniques as well as by immunocytochemistry of galactocerebroside (GC), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP). Preliminary results show that implanted cells seem to migrate from the implantation site to the lesion and that implanted-OL express myelin markers. Transplanted animals show an increase in the number of OL and a higher myelination rate as compared to saline-injected-control animals . Those results seem to indicate that transplanted OL survive and produce myelin in the host's demyelinated spinal cord and that the development of new strategies in the study of demyelinating diseases. (Supported by TIS 87/873, 88/1547 and Severo Ochoa-Ferrer Foundation award to JJLL).
STUDY OF NEURAL REGENERATION AFTER TRANSPLANTA-
TION: USE OF PHASEOLUS VULGARIS LEUCOAGGLUTININ AS
A CELL MARKER AND THE EFFECT OF DIHYDROERGOTOXINE
MESYLATE. H. Kamotani, K. Shimogami, T. Akagishi, K. Kimura
and J. Kimura (SPON: H. Ando). Dept. of Neurology, Kyoto Univ.,
Kyoto and Shiga Univ. of Medical Science, Shiga, Japan.

We present a Phaseolus vulgaris leucoagglutinin (PHA) method for
marking for delineated transplanted cells and their sprouting pro-
cesses. Cultured neural tissues or cell suspension prepared from embryonic rat
brains were marked by brief incubation with PHA and then transplanted
into the striatum or the cortex of nonimmunosuppressed young adult rats.
At various intervals (up to 2 months) after transplantation, animals were
fixed under deep anesthesia and serial cryostat sections were examined.
Transplanted neurons and their presumably regenerated processes were
successfully demonstrated by their immunoreactivity. Thus, PHA can be
used as a marker for delineating transplanted cells and their sprouting
processes. PHA has the following merits as a marker in neural trans-
plantation: First, PHA is readily taken up by neurons just prior to trans-
plantation. Second, it is retained for a more prolonged period than HRP.
Third, it is transported anterogradely and reflects the post-transplantation
growth of the neuronal processes.

Neural transplants with PHA marking can also be applied as a
model to study neural regeneration or evaluate in vivo effects of various
types of neurologically active substances on the neurite growth.

We used the method to study the effect of dihydroergotoxine mesylate on
the transplanted neurons.

ANALYSIS OF INTRASPINAL NITROCELLULOSE FILTER IMPLANTS.
F. J. Luesli and B. Tedeschi*. Eastern Virginia Medical
School, Norfolk, VA 23501.

Implants inserted into the mammalian spinal cord of neo-
tal astrocyte-coated filters have been reported to dimin-
ish host response to injury and provide a substrate for
axonal growth (Silver, CNS Trauma Sym., 1987). To see if
this study was to evaluate, using EM, the influence of
filter implants on host adult rat spinal cord. Following
avulsion of L3,4 and 5 dorsal roots of three types of
filters were implanted within a slit along the dorsal-
ateral sulcus after which the roots were tucked medially
along the sulcus. Survival times were up to 2 months. The
three filter types were: 1) uncoated; 2) polylysine-coll-
gen coated; and 3) astrocyte-coated (cells harvested at
from E15 or at birth). The first two types of filters
evolved similar host responses. A connective tissue matrix
(CTM) formed around the filters as did a new glia limi-
tans. At a level in which the dorsal roots were labeled
with HRP, regenerating axons were seen in CTM around
the filters and occasionally within the cord. At the EM
level, Schwann cell myelinated axons were observed within
the CTM but not on or immediately adjacent to the filters.
Filters coated with astrocytes harvested at E15, were
also surrounded by a CTM and were devoid of survi-
ving astrocytes. Implants with newborn astrocytes are cur-
cently being analyzed. Supported by NIH Grant NS24309,
SCRF (FIS), and Institutional Grants (FIS, BMT).

IMMUNE REACTIONS IN HOST RATS AGAINST INTRACEREBRAL GRAFTS
OF MOUSE HIPPOCAMPUS AND MOUSE GLIAL CELL CULTURES.

(SPON: M.I. West). Pharmacol. Unit, Inst. of Neurobiology
and Z/nat. of Microbiology, Univ. Aarhus, Aarhus, Denmark.

Rejection of fetal CS/mouse hippocampal xenografts in-
serted bilaterally into the hippocampal region of adult
Kyoto rats is usually completed after 5 weeks. The cellu-
lar infiltration of the grafts increased up to 3 weeks
postgrafting. The infiltrate consisted mainly of macro-
phages and T-cells of the Ts/c and NK-cell phenotype. Anti-
phages and T-cells of the Ts/c and NK-cell fenotype. Anti-

g strains were used to evaluate the effect of trauma on the concentra-
tion of MHC Class II antigens in the mouse brain is due to factors unknown to us. Supported by
NSF BNS-8112892 and NIH BRSG 2 S07 RR 0762.

LAMIN INJECTION CREATES TRAUMA: SCIENCE DIRECTS AND
GUIDES TISSUE IN-growTH. E. Garcia-Hernandez, F.C. Zhou,
and J.C. Azmitia. Dept. of Biology, New York University, NY, NY 10003.

Lamin direct outgrowth of transplanted (TP) neurons into adult
hippocampus (HIP) (Zhou and Azmitia, J. Chem. Neuroanat., 1:133,
1988). Does lamin guide fetal neureticles into the aged
and regenerating sprouts in the adult brain? For TP studies, adult and aged
2-5D male rats (2-3 months) were perfused intracardially with the MFB to
resect the hippocampus; 3-5HT neurons were found after I
weeks postgrafting. γ-interferon stimulation
3 weeks postgrafting. Cytotoxic antibodies
were also found after 1 week postgrafting. Gamma interferon stimulation
of fetal 5-HT neurons bilaterally into the HIP. A laminin tract was
made 0.5 mm lateral from the TP site. Adult female rats were
injected with 5-HT, then stained with anti-5HT (5-HT-TRITC) and the
and 14 days later, a laminin tract was made from the
lesion site toward the ventral hypothalamus. 5-HT immunocyto-
ichemistry was done after two weeks. Dense and straight fibers
from the graft tissue were seen within the laminin tract of adult,
but not aged animals or vehicle tracts. 5-HT-TRITC-induced sprouts
elongated toward the laminin tract. The absence of the elongation
in the aged brain is due to factors unknown to us. Supported by
NSF BNS-8112892 and NIH BRSG 2 S07 RR 0762.

INTRACEREBRAL IMPLANTATION OF IONOGENTIC MATRICES.
S. Weeley*, R. Marchand, C. Layville* and B. Rudoin*.

Charlotte, NC 28203.

The introduction of an extracellular matrix-like architecture, an arti-

culture of miotic and permanent scaffolding structures for brain wounds, may
assisting tissue ingrowth and regeneration. Synthetic hydrogels of poly-

gency of methylcellulose (poly GMA) are well tolerated by brain tissue
and, depending on pore characteristics, allowed ingrowth of tissue (Woerly et al.,
Neurosci. Lett. 123, 370-373, 1988). Here, the effect of ionogenic groups
incorporated into polyGMA hydrogels on the reactivity of the lesioned
neural tissue was studied since regenerating axons may respond to charges
in size and in number. Charged polyGMA were obtained by
polymerization of a monomer solution containing either basic groups
with diethylenamino-ethyl methacrylate (DREAMA) or acid groups with
methacrylic acid (MAA). Collagen was adsorbed into the polymers
to give the necessary bioadhesivity. SEM showed matrices of polyGMA-
DREAMA with pores of 10μm while matrices containing MAA had
heterogeneous porosity. Samples were implanted into the parietal cortex
of adult rats, and after 3 months, the brains were examined in histology
(cresyl, PAS, Bodian) and for GFAF. The implants caused a variable
astrocitary reaction. Astrocitary processes contacted the gels and some
entered. Extracellular material (reticulin and PAS substances),
capillaries and heterogeneous cells accumulated at the brain-implant
interface and reticulin was deposed into the polymers. Nerve fibers
grew marginally in the tissue, some crossing the interface and a few
projecting onto or into the matrices. Matrices carrying charged groups
increase the diameter of the wound healing (MRC, FRSQ & PCAR).

CHANGES IN PERIPHERAL NERVE MHC CLASS II ANTIGENS
FOLLOWING TRAUMA. T. E. Trumbi*, J. Stanislaw*, R.
Jacobson*, R. Troland* (SPON: C. Duncan). Dept. of
Orthopededics and Rehabilitation, Yale Univ. School of
Med., New Haven, CT 06510.

As a prelude to the transplantation of peripheral nerve tissue, human peripheral nerve tissue samples were analyzed
for the presence of MHC Class II antigens and a mouse model
was used to evaluate the effect of trauma on the concentra-
tion of MHC Class II antigens. Methods Part I: 1) autopy and 5 operating room (OR) specimens were
removed from individuals without systemic disease. The specimens
were frozen with liquid nitrogen, OCT implanted, stained
with equine anti-mouse IgG biotinylated secondary Ab and
avidin-DH-horse radish peroxidase H complex followed by
3,3 diaminobenzidine. Part II: 10 C5H-hej mice underwent
the nerve distal to the transsection site and the contra-
averopelvicin line and the contra-

neural regeneration, the concentration of MHC Class II antigens in the mouse

neural regeneration, the concentration of MHC Class II antigens in the mouse

neural regeneration, the concentration of MHC Class II antigens in the mouse

neural regeneration, the concentration of MHC Class II antigens in the mouse

neural regeneration, the concentration of MHC Class II antigens in the mouse

Measurable Properties of Grafted Neurons and Potential Relationship to the Blood-Brain Barrier (BBB)


Depending on placement, the BBB to protein in neural grafts can be variably permeable. Being exposed to the circulation, graft neurons' metabolic activity could also alter from the normal. Young adult rats received 15T of paraineloplastical IP grafts of fetal neocortex from 191-19 donors. The grafts were examined on 14 days after transplantation. The grafts were examined by immunocytochemistry using an antibody against AA, a marker for adult rat vasculature. The grafts were also observed for changes in BBB permeability. The results showed that the BBB to protein in grafts was variably permeable. The permeability of the BBB was dependent on the donor and host species. The permeability of the BBB was also affected by the presence of endothelial cells. The results suggest that the BBB to protein in neural grafts can be variably permeable, and that the permeability of the BBB is dependent on the donor and host species.

Neurogenic Reorganization and Plasticity Following Fetal Hypothalamic Transplantation into the Mammillary III Cranial Ventricle

M. B. Bechard and S. W. Miller, Department of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501.

This investigation deals with neurovascular organization and fetal neural grafting into the third cranial ventricle of 15 host rats. Fine capillary networks were examined in grafts within the first week following transplantation. Most capillaries in fetal grafts were observed. Limited numbers of fenestrated capillaries were found at the periphery of grafts, underlying the host median eminence. Axon profiles filled with collagen were observed in vessels. These results indicate that maintenance of fetal neural grafts is essential. The results suggest that the BBB to protein in neural grafts can be variably permeable. The permeability of the BBB was dependent on the donor and host species.
540.3
IN VIVO DOPAMINE (DA) RELEASE FROM THE CAUDATE NUCLEUS (CN) OF FEMALE RATS IN RESPONSE TO L-DOPA VARIES WITH THE ESTRUS CYCLE.
D. E. Dusek and V. D. Ramirez, Department of Physiology and Biophysics, University of Illinois, Urbana, Ill. Intact female rats (N=5) with regular four day estrous cycles were implanted with a push-pull cannula directed at the CN and were perfused on each day of their estrous cycle. Perfusate samples (6-8 ul/min) were collected at 15 min intervals and assayed for DA using HPLC-EC. Following a one hour basal collection period, L-DOPA, infused in perfusion medium, was infused directly into the CN through the push cannula at two increasing doses (1 and 10 uM) during each of the stages that comprised the basal (15 min) DA release. Basal DA release was significantly reduced from the CN of freely moving animals, indicating the importance of the endocrine milieu in the function of the nigrostriatal dopaminergic system.

540.4
RIGHT-LEFT ASYMMETRY OF TYROSINE HYDROXYLASE (TH) ACTIVITY IN RAT MEDIAN EMINENCE (ME): INFLUENCE OF BAROREFLEX NERVES.
S. Alexander, T. N. Kaneda, A. Ishii, M. Mogi, M. Harada, K. E. Moore (SPON: J. L. Bennett). Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824. The basal level of activity of TIDA neurons is higher in female than in male rats. Furthermore, restraint stress increases plasma levels of prolactin in both male and female rats, but only in females does this increase appear to be mediated by brain TH activity.

540.5
TRADITIONAL PROPYLETHIOUARIC ACIDS USED FOR INDUCING HYPOTHYROIDISM ARE TOO HIGH. T. T. Sherer* and R. J. Hul, Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman, WA 99164.
Propylthiouracil (PTU) is commonly used in experimental models to induce a hypothyroid state. However, information on the minimum doses required to maximally suppress thyroid function of fetal and neonatal models is not available. To obtain appropriate dose-response information pregnant Sprague-Dawley rats were exposed to various levels of PTU (0, 50, 100 and 200 mg/kg) in the drinking water from gestational day 20 to postnatal day 21. Litters were collected from 2 pups per litter on postnatal days 1, 6, 12 and 21. Purkinje cell arborization observed in neonates at doses at least 10-fold less than what has been commonly used in past studies. (Supported by NASA Grant No. NAG 9-276.)

540.6
Previous studies have demonstrated that the sympathetic hypogastric ganglion (HG) is dependent upon the continued presence of testosterone for normal maintenance of tyrosine hydroxylase (TH) mRNA, protein, and activity. The present study establishes that TH mRNA and TH activity in the HG of castrated rats are reduced. The reduction in TH mRNA was examined further by in situ hybridization using a ribonuclease synthesized from a fragment of TH cDNA which has been inserted and immunocytochemistry with a TH-specific polyclonal antibody to determine whether specific subpopulations of neurons within the HG are responding to castration. The results support the finding that TH mRNA levels become reduced following castration and reveal that the reduction is restricted to a specific TH-positive population of cells in the HG. Therefore, we have further investigated whether testosterone is acting directly on the TH gene, or acting indirectly by affecting nerve growth factor (NGF) levels in a major target of the HG, the vas deferens (VD). Double analysis of NGF mRNA in the VD reveals a progressive decrease in NGF mRNA in the VD 1, 2, 4 and 8 weeks following castration and levels are restored following treatment with testosterone. It is possible that TH levels in the HG are dependent upon the continued presence of NGF in the VD which is in turn dependent upon the continued presence of testosterone.

540.7
FURTHER STUDIES ON LHRRH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE (NE), C.A. Barral, H. D. Hartman, Dept. of Physiol., Univ. Maryland, Sch. of Med., Baltimore, MD 21201.
Previously, we reported that LHRH neurons of estrogen-treated, ovariectomized rats are severely limited in their responsiveness to NE. In the present study, we examined the combined effects of the sympathetic preganglionic nerve (pGEP) and NE on LH release. Plasma LH increased during ES to reach peak values rapidly thereafter. ICV NE infusion directly into the ME at the CN, was infused directly into the CN through the push cannula at two increasing doses (1 and 10 uM) during each of the stages that comprised the basal LH release. Basal LH release was significantly reduced from the CN of freely moving animals, indicating the importance of the endocrine milieu in the function of the nigrostriatal dopaminergic system.

540.8
EFFECTS OF ORCHIDECTOMY ON BASAL AND STRESS-INDUCED DECREASES IN TUBERINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONAL ACTIVITY. T. W. Toney, J. K. Jorlingdon and K. E. Moore (SPON: J. L. Bennett). Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.
The basal level of activity of TIDA neurons is higher in female than in male rats. Furthermore, restraint stress increases plasma levels of prolactin in both male and female rats, but only in females is this increase accompanied by stress-induced decrease in TIDA neuronal activity. In the present study TIDA neuronal activity was estimated by measuring the administration of a decarboxylase inhibitor. One and two weeks following orchidectomy, dopamine (DOPA) accumulation was increased in the median eminence of castrated rats. The reduction in TH mRNA was examined further by in situ hybridization using a ribonuclease synthesized from a fragment of TH cDNA which has been inserted and immunocytochemistry with a TH-specific polyclonal antibody to determine whether specific subpopulations of neurons within the HG are responding to castration. The results support the finding that TH mRNA levels become reduced following castration and reveal that the reduction is restricted to a specific TH-positive population of cells in the HG. Therefore, we have further investigated whether testosterone is acting directly on the TH gene, or acting indirectly by affecting nerve growth factor (NGF) levels in a major target of the HG, the vas deferens (VD). Double analysis of NGF mRNA in the VD reveals a progressive decrease in NGF mRNA in the VD 1, 2, 4 and 8 weeks following castration and levels are restored following treatment with testosterone. It is possible that TH levels in the HG are dependent upon the continued presence of NGF in the VD which is in turn dependent upon the continued presence of testosterone.
Several lines of evidence suggest that somatostatin (SRIF) may directly interact with GH-releasing factor (GRF)-containing neurons, centrally to modulate GH secretion. To determine whether such interactions are implicated in regulating GH content of arcuate (ARC) neurons, and consequently GH secretion, we examined the effects of the potent SRIF-depleting agent, cysstemine (CSH), on ARC GRF immunoactivity and on pulsatile GH secretion. Serial sections of hypothalami of CSH-treated rats studied with PAP immunocytochemistry demonstrated a striking increase in both the number (126%) and staining intensity of immunoreactive cells compared to age-matched controls. This increase was significant (P<0.01) at all levels of the ARC nucleus but most pronounced within the caudal tier. Administration of CSH (90 mg/kg i.v.) to normal adult rats significantly increased plasma GH levels (P<0.01) which was prevented by passive immunization with a specific antiserum to CSH (2.8 ± 0.8 vs. 9.3 ± 2.0 ng/ml in normal serum vs. CSH-injected controls; P<0.02) indicating a role for endogenous GH. Together, these findings support the concept that SRIF exerts a tonic inhibitory influence on GRF-containing ARC neurons and may play an important role in the physiological control of pulsatile GH secretion.

Human growth hormone response to ovine corticotropin releasing factor (CRF) was prevented by passive immunization with a specific antiserum. Since the effects of the antiserum injection were opposite to those of ovine CRF, the latter is shown to exert a tonic inhibitory influence on GRF-containing ARC neurons. Together, these findings support the concept that SRIF may directly interact with GH-releasing factor (GRF) neurons. GH concentrations were measured in 15 minute blood samples taken before and after injections which were in 0.25 µg/g. Average basal concentrations of GH were increased by injection of the mu agonist D-Ala2-Gly-o1 Enkephalin (DAGO) at a minimum effective dose of 0.0001 nanomole. The maximum effective dose of the delta agonist D-Pen2,D-Pen5 Enkephalin (DPPE) and kappa agonist USO,488-H was 1.0 nanomole, GH secretion caused by DAGO was blocked by systemic pretreatment with naloxone 5 mg/kg. We conclude that the mu opioid receptor activation on or close to GRF neurons causes GH secretion. The effectiveness of 10,000-fold higher doses of DPPE and USO,488-H in stimulating GH suggests that they may be non-specifically activating mu receptors. Supported by grants NS 19266 and MTA (OKTA 104).

Growth hormone (GH) is released by systemically administered opioids and several opioid receptor subtypes have been implicated. We prepared anesthetized male albino rats in standardized fashion. Blood samples were obtained at -15, 0, +15, +30, +45, +60, +90, +120, +180, and +210 minutes. Samples were analyzed for cortisol, ACTH, and growth hormone (GH) and delayed compared to those of cortisol and ACTH. The average peak response for those controls who did not respond was 5.11 ng/ml and this peak occurred 180 minutes after the CRF bolus. The average peak response for responders in the depressed group was 3.89 ng/mL and occurred 120 minutes after the CRF bolus. These results support the observation that the pituitary of depressed patients is dysregulated compared to normals.

Supported by NIMH grants MH 42038 and MH 39593.
Presence of Galanin-like Immunoreactive Material in the Hypophyseal Portal Blood of the Rat. J.I. Koenig and S.M. Gabrieli. Neurology Service, Massachusetts General Hospital, and Harvard Medical School, Boston, MA 02114 and Department of Psychiatry, Mount Sinai School of Medicine, New York, New York 10029.

Recent studies have demonstrated that galanin (GAL) may play a modulatory role in episodic growth hormone (GH) secretion in the rat. GAL appears to act in the hypothalamic area to modulate pulsatile release of GH secretion. Furthermore, GAL is found in high concentrations in the median eminence in close proximity to the hypophysial portal capillary loops. These studies support the notion that GAL may be secreted into the portal vasculature. In the present study, we have sought to determine if GAL is present in the hypophysial portal circulation. Adult male rats were anesthetized with urethane or pentobarbital.

Hypophysial portal blood was collected as previously described (Dolencroxy 1981:25:1). The concentration of GAL in the portal plasma was determined using an antibody developed against rat GAL conjugated to KLH with 540.15

CHARACTERIZATION. M. A. Sortino. F. Nicoletti, and P. L. Canonicco. Department of Pharmacology, University of Catania, Italy.

Excitatory amino acids stimulate inositol phospholipid hydrolysis in slices prepared from rat hypothalamus. As occurs in other brain regions, this stimulation is in each group during the first 15 days of postnatal life (up to 15 fold increase with quisqualate or ibotenate). Progressive of declines during maturation, is present after 30 days but disappears in slices from 2 month old animals. In hypothalamic slices, quisqualate is by far the most potent activator of inositol phospholipid hydrolysis (EC50 value = 2-5 uM), followed by ibotenate and glutamate, whereas N-methyl-D-aspartate (NMDA), kainate and amino-dydroxymethylisoxazolopropionate (AMPA) are inactive. In hypothalamic slices from newborn animals, maximal stimulation of [3H]inositol monophosphate formation by quisqualate, ibotenate and glutamate is greater than that induced by noradrenaline or carbamylcholine. Hypothalamic slices are a suitable model to study whether excitatory amino acid receptors regulate the pattern of neurohormone secretion during maturation.


We have previously demonstrated that stereotactic infusion of kainic acid (KA) into the lateral preoptic or the medial septal area effectively blocks estrogen and progesterone stimulated luteinizing hormone secretion (an E + P induced LH surge) in addition to causing parietal hyperthermia (Clough, et al. 1981: Biol. Reprod. 35: 189-196). It was presumed that these effects were a result of the excitotoxic effect of KA on neurons in the infarction area, including LHRH neurons. The present study has used a perfusion culture system to determine the effects on KA on LHRH release from organotypic preoptic-medial septal hypothalamic (PO-MBH) explants. KA was perfused from femoral catheters and were placed individually into 200 ul chambers housed in a gas and heat exchanger. The KA was then perfused with warm and oxygenized Glucose-F12 medium at a rate of 25 ul/min. At 0.5, 1, and 6 h perfusion, explants were exposed to culture medium containing 1 uM KA. LHRH release over time was assessed by radioimmunoassay. Release of KA caused an acute increase in LHRH release into the perfusate fluid followed by a gradual decline to sub-baseline levels. Exposure of explants to potassium chloride, subsequent to KA release. In other studies, we have demonstrated KCl responsiveness of PO-MBH explants in LHRH neuron destruction, this study does not suggest that E + P act directly on LHRH neurons to induce an LH surge. Rather, this study suggests the LHRH neuron as the final common pathway to the adenohypophyseal gonadotrophs. Supported by HD 24425 (RWC).
541.1 TEMPORAL AND SPATIAL DISTRIBUTION OF A DROSOPHILA PROTEIN WHICH RESEMBLES BETA-AMYLOID PRECURSOR. G.M. Cole, T. May shed only the extracytoplasmic domains of the extracytoplasmic domain (P2; Tate-Ostroff Antibodies are to the N terminal region (N1), and the Sandoz Foundation. Supported by AG02126, AHAF the location of APP domains in PC12' Alzheimer whole cells transfected with the same APP fragment, is also toxic to neurons in primary rat hippocampal cultures. We tested the generality of these phenomena by determining the effect of ABI CM from APP-transfected on the human neuroblastoma cell line LAN-5 which had been induced to differentiate with 25% retilicin acid (RA). ABI CM was added to LAN-5 cells after 4 d in culture with RA. 50-75% of the RA-treated cells died after 5-6 d in ABI CM. In contrast, LAN-5 cells in CM from transfected cells differentiated normally in response to RA. The generative effect of the ABI CM depended on the degree of differentiation achieved by LAN-5 cells before the addition of ABl medium. Our data suggest that the neurotoxic activity of the APP ABl fragment on rat PC12 cells induced to differentiate with NGF and the normal neurotrophic factor for PC12 cells, is not dependent on NGF. 

541.3 IMMUNOCYTOCHEMICAL STUDIES OF BRAIN AND PC12 CELLS USING ANTIBODIES TO THE AMYLOID PRECURSOR PROTEIN R.C. Marotta, X.G. Wecott, B. Haiduc, R. Wolanski, L. Mao, B. And P. L. Majocha, L. M. Matthysse) Mass Gen Hosp, Harvard Med Sch, Boston, MA; McLean Hosp, Belmont, MA and R. L. Neve, The Children's Hospital, Boston, MA. We developed antibodies to APP domains for immunostaining of human brain and PC12 cells. Antibodies are to the N terminal region (N1), the extracytoplasmic region (P2; Tate-Ostroff et al., PNAS 86:745; 1989), the P-beta region (A4; Majocha et al., PNAS 85:6182; 1988) and the C terminal region (C1). Brain and PC12 cells stained with N1, P2 and C1 antibodies. Alzheimer brain showed N1, P2 and C1 stainings associated with thioflavin positive plaques. Conditioned media from normal PC12 cells was found to inhibit the staining of brain tissue by all APP antibodies except A4 and C1, indicating that PC12 cells may shed only the extracytoplasmic domains of the APP. These studies provide insight into the location of APP domains in PC12, Alzheimer and control brains, as well as the processing of the molecule. Supported by AG02126, ARAF and the Sandoz Foundation.

541.4 REGULATION AND RELEASE OF AMYLOID AMYLOID PRECURSOR PROTEIN IN MEMBRANE DAMAGED NEURAL CULTURES. F. Banik*, R.N. Rosenberg, and S.A. Stein. Dept. of Neurol., Univ. Texas Southwestern Medical Center, Dallas, Texas. We developed antibodies to APP domains for immunostaining of human brain and PC12 cells. Antibodies are to the N terminal region (N1), the extracytoplasmic region (P2; Tate-Ostroff et al., PNAS 86; 745; 1989), the P-beta region (A4; Majocha et al., PNAS 85:6182; 1988) and the C terminal region (C1). Brain and PC12 cells stained with N1, P2 and C1 antibodies. Alzheimer brain showed N1, P2 and C1 stainings associated with thioflavin positive plaques. Conditioned media from normal PC12 cells was found to inhibit the staining of brain tissue by all APP antibodies except A4 and C1, indicating that PC12 cells may shed only the extracytoplasmic domains of the APP. These studies provide insight into the location of APP domains in PC12, Alzheimer and control brains, as well as the processing of the molecule. Supported by AG02126, ARAF and the Sandoz Foundation.

541.5 SECRETION OF AMYLOID-B PROTEIN PRECURSOR. G.M. Cole, T. Mayhew*, D. Schubert*, and T. Sanchez (SPON: W.C. Wiederhold). University of California, San Diego, Dept. of Neurosciences, M-024, La Jolla, CA 92033, Salk Institute for Biological Studies, La Jolla, CA 92037, U.S.A. We reported part or all of amyloid-b protein precursor (ABPP) is released into the conditioned medium (CM) of PC12 and other cells (Schubert et al., Science 241:229-228, 1988; Schubert et al., PNAS 86:2066-2069, 1989; Ueda et al., J Neurosci 15:245-251, 1989). Both the insert and non-insert immunoreactive forms of ABPP are secreted, but C-terminal antisense reacts only with the membrane portion of ABPP. The size of the secreted ABPP is similar to the size of the full length membrane form which suggests ABPP is normally shed in or near the amyloidogenic A4A region which is next to the membrane. Here, we show the majority of ABPP in the CM of human neuroblastoma, teratocarcinoma, melanoblastoma and retinoblastoma and glioma cells demonstrating that ABPP is generally secreted. Treatment of cells with leupeptin, chloroquine or ammonium chloride dramatically increases the soluble intracellular and extracellular forms of ABPP suggesting a possible lysosomal pathway in ABPP metabolism. With lysosomal protease inhibitors similar increases are also seen in a small C terminal immunoreactive membrane fragment suggesting it may also undergo lysosomal processing. Experiments are underway to determine the relationship between normal secretion and lysosomal processing.

541.6 ROLES FOR INTERLEUKIN-1 AND NERVE GROWTH FACTOR IN AMYLOID FORMATION IN ALZHEIMER'S DISEASE. F. Banik*, D. Casper, R. Heilandt, V. Friedrich, M. Belof, R. Lindblom, M. Blum, and N. Robakis. Fishberg Res. Center in Neurobiology, Dept. Psychiatry and Molecular Biology, Mount Sinai School of Medicine, New York, New York 10029. Neuronal growth factor (NGF) can induce secretion and can alter processing of beta-amyloid precursor protein (APP) in several cell lines (Refolo et al., submitted). These neuronal interleukin-1 (IL-1) in turn can release NGF production (Lindholm et al., Nature 330,658 (1987)). Moreover, IL-1 also can regulate APP mRNA levels, as can be concluded from our study showing increased APP mRNA levels in hippocampal neurons in response to IL-1. These studies suggest the existence of an IL-1-NGF cascade regulating synthesis and maturation of APP. In order to investigate whether such a cascade operates in CNS tissue, we cultured hippocampal cells and frontal cortex tissue from the rat E18 fetus. Interestingly, in the same area, we found by in situ hybridization analysis using a 35-mer IL-1 beta oligonucleotide, abundant cells expressing IL-1 mRNA in the adult rat. Since hippocampal and cortical cultures contain neurons and glia, we also established cultures of glia alone. All cultures were characterized by immunostaining using antibodies to neurofilament (neurons), glial fibrillary acidic protein (astrocytes), galactocerebroside (oligodendrocytes), O4 and A235 (glial precursors). In the hippocampal cultures, IL-1 increased mRNA levels coding for NGF. Western blot analysis with different antibodies to APP showed the presence of APP in hippocampal and glial cultures. In neurons the APP staining was distributed over the cell bodies and neurites. In glial cells, APP staining was present in astrocytes type 1 and appeared to be associated with the cytoskeleton of the cells. Experiments to determine whether an IL-1-NGF cascade controls APP production and processing are in progress.
TRANSFERENCE OF $\beta$-AMYLOID INTO CHOLINERGIC NEURONAL CELL LINES. D. Galasko*, G. Cole*. T. Lai*. T. Saieh.* (Dept. Neurosci., UCSD, La Jolla, CA 92039; *Neurology Ser., VA Med. Ct., San Diego, CA 92161)

Beta protein, a 4.2 kD polypeptide, is a major component of amyloid deposits in Alzheimer's disease (AD). Three MrNA sequences for amyloid beta protein precursor (ABPP) molecules of 665, 751 and 770 amino acids. ABPP is proposed to be an integral membrane protein with a short transmembrane domain, similar to a membrane domain containing part of the beta protein sequence, and a large extracellular N-terminal domain, which contains an insert region, a proteinase inhibitor, in the 751 and 770 amino acid forms. To obtain an index of ABPP metabolism in the brain, we measured ABPP immunoreactivity in human cerebrospinal fluid (CSF), by immunostaining Western blots. A polyclonal antibody to an amino acid sequence in the N-terminal region of ABPP stained bands of approximate Mr 88 and 100 kD. These two bands corresponded to similar bands in blots of conditioned medium from Alzheimer and Down Syndrome patients. This protein is expressed in the brain and a wide variety of tissues. The application of these antibodies resulted in specific staining which in some cases differed from mRNA studies. Pretreatment of tissue with proteinase K was required to produce significant staining (with 291 and 612, while the use of proteinase K pretreated glutaraldehyde fixed tissue resulted in striking cellular staining with 324).

We will present data on the distribution of the antibodies in aged and young rats. Further we are investigating the role of ABPP in the regulation of subtypes of APP.


TEMPORAL EXPRESSION OF AMYLOID-BETA-PROTEIN mRNA IN HIPPOCAMPAL NEURONS IN VITRO. N.L. Strong*, A. Sweeney, K.N. Garraulo, G.J. Gajdusek. LCAS, NINDS, NIH, Bethesda, Maryland 20892.

The origin of the amyloid-beta-protein deposited in the neuritic plaques of Alzheimer's disease is unknown. We have investigated the potential for a neuritic origin and have evaluated the temporal expression of amyloid precursor protein (APP) in hippocampal neurons in culture. Dissociated hippocampal neurons from New Zealand white rabbits at fetal day 29-30 were cultured in defined medium over confluent mature astrocytes. In situ hybridization with a biotinylated riboprobe transcribed from a cDNA encoding the amyloid precursor protein was performed at intervals to 30 days in culture. Age matched cultures were hybridized with a sense probe as controls. Prior to the development of features of neuronal maturity, APP mRNA could be localized to the neuronal perikarya and proximal neuritic tree. As the neurons reached maturity, signaled by the expression of all-neurofilament subunit proteins and by the acquisition of morphological features of hippocampal neurons in vivo, the APP mRNA localized to the perikarya alone. Our findings demonstrate the expression of APP mRNA in high, both sending neurons in vitro and also suggest that this expression is developmentally regulated. (Supported in part by the Medical Research Council of Canada)


Although the beta-amyloid peptide is an established core component of neurtic plaques that accumulate in Alzheimer's disease, the mechanisms responsible for its deposition are not well understood. We now report that lesions of rat hippocampal neurons caused a time-dependent, long-lasting elevation of immunoreactivity for the beta-amyloid precursor protein (APP) in neighboring astrocytes, all of which normally express the protein in hippocampus. Neuronal damage produced by intraventricular kainate or colchicine was paralleled by stab wounds that cause the alternate APP mRNA accumulation. The increase represented astroglial expression of the protein rather than a scavenging of APP released by damaged neurons. Immunoelectron microscopy confirmed that APP-containing cells surrounding capsulated lesions were reactive astrocytes and within the neuropil. Antibodies directed against either the amino- or carboxy-terminal of APP labelled reactive astrogia, consistent with expression of a full length precursor by these cells. The results demonstrate that neuronal damage stimulates APP expression in adult brain, and suggest that reactive astrocytes may be a source of the beta-amyloid that forms neuropathological plaques in Alzheimer's disease.

LOCALIZATION OF beta-AMYLOID PRECURSOR PROTEIN IN REACTIVEastrocytes FOLLOWING NEURONAL DAMAGE. B. Simon, J.P. Card, R.B. Nelson and J.G. Davis. Medical Products Dept., The DuPont Co., Wilmington, DE. 19880-0400.

Although the beta-amyloid peptide is an established core component of neuritic plaques that accumulate in Alzheimer's disease, the mechanisms responsible for its deposition are not well understood. We now report that lesions of rat hippocampal neurons caused a time-dependent, long-lasting elevation of immunoreactivity for the beta-amyloid precursor protein (APP) in neighboring astrocytes, all of which normally express the protein in hippocampus. Neuronal damage produced by intraventricular kainate or colchicine was paralleled by stab wounds that cause the alternate APP mRNA accumulation. The increase represented astroglial expression of the protein rather than a scavenging of APP released by damaged neurons. Immunoelectron microscopy confirmed that APP-containing cells surrounding capsulated lesions were reactive astrocytes and within the neuropil. Antibodies directed against either the amino- or carboxy-terminal of APP labelled reactive astrocytes, consistent with expression of a full length precursor by these cells. The results demonstrate that neuronal damage stimulates APP expression in adult brain, and suggest that reactive astrocytes may be a source of the beta-amyloid that forms neuropathological plaques in Alzheimer's disease.


The beta-amyloid peptide is found as a core component of neuritic plaques in Alzheimer's disease, and is contained within a family of precursor proteins (APP) of predicted sequences from cDNA clones. However, APP is of unknown structure and function. We have raised antibodies to a variety of peptide domains from this protein and used Western blot analysis to detect, characterize and partially purify APP from rat brain. Carboxy-terminal antibodies recognized a triplet of APP polypeptides of Mr-110-130 KD. These polypeptides were exclusively associated with membranes, but could be selectively extracted in low ionic strength buffer, a property characteristic of extrinsic membrane proteins. APP appears not to be glycosylated, since it neither bound to various types of lectin-conjugated Sepharose beads, nor was susceptible to glycosidase-mediated alteration in gel migration. APP was purified more than 2000-fold by immuno-affinity chromatography, gel filtration, and heparin-arsonase affinity chromatography. Through these procedures, two of the APP polypeptides were purified, while the third was differentially separated by the anion exchanger.


Since the beta-amyloid peptide is contained within a family of precursor proteins (APP), proteolytic processing of APP is required for beta-amyloid peptide formation. We have begun to investigate mechanisms of APP processing, using two approaches. First, immunoblotting with APP antibody was used to assess the in vitro degradation of rat or human brain APP and APP-like proteins by APP. Second, the localization of APP was compared with that of several proteases by immunocytochemistry. On Western blots, antibodies to the C-terminal domain of APP recognized three APP polypeptides in rat and human brain membranes, of Mr-110-130 KD. Both rat and human APP were extensively sensitive to a variety of proteases, including the calcium-activated protease calpain I, trypsin, papain, and cathepsin G. In particular, calpain I degraded APP under conditions in which few other proteases were affected. Throughout rat brain, calpain I and APP immunoreactivities were confined to the same neuronal populations, with especially high levels of each in olfactory bulbs, layer 5 of the cerebellum, subiculum, globus pallidus, red nucleus, and cerebellar Purkinje cells and deep nuclei. Intraventricular kainate infusion, which causes rapid activation of hippocampal calpain I, did not produce substantial neuronal loss or seen affected and both APP and calpain I immunoreactivities were observed in the surrounding reactive astroglia. Collectively, these results indicate that calpain I may be involved in the normal and, perhaps, pathological processing of APP.
ELEVATION OF AN AMYLOID PRECURSOR mRNA IN ALZHEIMER'S DISEASE AND DOWN SYNDROME. R. L. Neve1 and C. A. Higgins.2
1Children's Hospital, Boston, MA 02115; 2University of Rochester Medical Center, Rochester, NY 14642.

At least three different alternately spliced mRNAs encode three variants of the Alzheimer amyloid precursor protein (APP). The sequence of events leading to the aberrant proteolytic processing of the amyloid precursor(s) may be linked to a change in abundance of expression of one of the forms of APP. To learn more about the relationship of APP mRNA expression and the development of AD and Down syndrome (DS) pathologies, we have undertaken a systematic analysis of APP mRNA expression in defined regions of normal and diseased brains. We have combined two methods of quantitative in situ hybridization and in situ hybridization and quantitative mRNA analysis of different APP transcripts. The relationship between APP gene expression by NGF in vivo, we have initiated studies in the basal forebrain using in situ hybridization and quantitative mRNA analysis. These results support the hypotheses that APP is extensively distributed, is deposited in terminal zones of synaptic density virtually all the neurons are killed, whereas the density of the cultures. At low plating density virtually all the neurons are killed, whereas the proportion of neurons surviving 4 days of exposure to AB1 CM increases at higher densities. Neurons cultured for one month in vitro are more resistant to the AB1 CM toxi­city, and may be cultured for two weeks in the presence of AB1 CM with minimal cell loss (comparable to control cultures grown in the presence of CM from nontransfected cells). The glutamate receptor antagonists kynurenic (1 mM) and 2-amino-5-phosphovalerate (APV, 100 uM) do not protect the neurons against the neurotoxicity of AB1 CM. Future studies are directed towards pharmacological interventions which may ameliorate the AB1 neurotoxicity, as well as towards the purification of the neurotoxic agent.

CHARACTERIZATION OF NEUROTOXICITY OF A FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR IN PRIMARY RAT HIPPOCAMPAL CULTURES. L. R. Dawe and R. L. Neve. The Children's Hospital, Boston, MA 02115.

Conditioned medium (CM) from PC12 and NIH 3T3 cell transfected expressing the carboxyterminal 105 amino acids (AB1) of the Alzheimer amyloid precursor (APP) is toxic to neurons in primary cultures of E18 rat hippocam­pus. With one month in culture, APP-expressing cells display the intense localization of lysosomal proteinases. We propose that dying neurons, which display an abnormal accumulation of lysosomes, are a major source of these enzymes. To investigate the relationship between localization of cephap­sin D (CD) and cephaptan B (CB), we used antibodies to human CB and CD and histoologically using Bichowskowsky silver stain and thioflavin S. Intact CD and CB immunoreactivities were consis­tently associated with SP that exhibited moderate or marked thioflavin positivity. These SP, identified as classical plaques, were most abun­dant within cortical layers III and V and layer II of the hippocampus. Silver positive 'primitive' type SP, which were distributed throughout all cortical and hippocampal laminae (particularly the molecular layer), did not contain prominent thioflavin staining, and were not highly immunostained with cephaptan antibodies. Our results suggest a strong relationship between prominent amyloid deposition extracellularly and the intense localization of lysosomal proteinases. We propose that lysosomal enzymes, released into the extracellular space from dying neurons, may contribute to the generation of amyloid and amyloid-related proteins within the NF of AD brain. Supported by NIA and AFAR.

NERVE GROWTH FACTOR (NGF) REGULATION OF AMYLOID GENE EXPRESSION IN AGED RAT FOREBRAIN. Gerald A. Higgins,1 Rachael L. Neve2, Karen S. Chen3 and Fred H. Gage.3 University of Rochester Medical Center, Rochester, NY 14642; 2Children's Hospital, Boston, MA 02115; 3University of California at San Diego, La Jolla, CA 92029.

Deficits in the NGF-responsiveness of basal forebrain cholinergic neurons may contribute to pathological changes in the aged CNS. One gene whose expression appears to be regulated by NGF is the amyloid precursor gene (APP), which encodes the Aβ protein component of amyloid deposits in aged and Alzheimer's diseased brain. In order to understand the regulation of APP gene expression by NGF in vivo, we have initiated studies in the basal forebrain using in situ hybridization and quantitative mRNA analysis of different APP transcripts. NGF receptor (NGF-R) and choline acetyltransferase (ChAT) mRNAs. NGF- or vehicle was infused into the striatum, and spatial memory was tested in a water maze task. In young adult rats, chronic NGF infusion produces robust increases in APP mRNA hybridization, NGF-R mRNA hybridization, and ChAT mRNA hybridization, and hypertyphy of ChAT mRNA-positive neurons. NGF treatment also increases the ratio of APP-695 mRNA to APP-751 mRNA in the basal forebrain. Aged rats with spatial memory deficits show increased levels of APP-751 mRNA in the forebrain as compared to aged non-impaired and young control rats. We are currently examining whether NGF treatment in aged animals can reverse changes in APP gene expression associated with behavioral impairment.

CHARACTERIZATION OF NEUROTOXICITY OF A FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR IN PRIMARY RAT HIPPOCAMPAL CULTURES. L. R. Dawe and R. L. Neve. The Children's Hospital, Boston, MA 02115.

Conditioned medium (CM) from PC12 and NIH 3T3 cell transfected expressing the carboxyterminal 105 amino acids (AB1) of the Alzheimer amyloid precursor (APP) is toxic to neurons in primary cultures of E18 rat hippocam­pus. With one month in culture, APP-expressing cells display the intense localization of lysosomal proteinases. We propose that dying neurons, which display an abnormal accumulation of lysosomes, are a major source of these enzymes. To investigate the relationship between localization of cephap­sin D (CD) and cephaptan B (CB), we used antibodies to human CB and CD and histoologically using Bichowskowsky silver stain and thioflavin S. Intact CD and CB immunoreactivities were consis­tently associated with SP that exhibited moderate or marked thioflavin positivity. These SP, identified as classical plaques, were most abun­dant within cortical layers III and V and layer II of the hippocampus. Silver positive 'primitive' type SP, which were distributed throughout all cortical and hippocampal laminae (particularly the molecular layer), did not contain prominent thioflavin staining, and were not highly immunostained with cephaptan antibodies. Our results suggest a strong relationship between prominent amyloid deposition extracellularly and the intense localization of lysosomal proteinases. We propose that lysosomal enzymes, released into the extracellular space from dying neurons, may contribute to the generation of amyloid and amyloid-related proteins within the NF of AD brain. Supported by NIA and AFAR.


Senile plaques showing a spectrum of morphologies in the cerebrum are known to be labeled by antibodies against synthetic or purified β amyloid protein (AP), also called Aβ. We now compare several antibodies raised to non-β regions of APP with antibodies to the C-terminus and N-terminus of APP recognized by some antibodies. We now compare several antibodies raised to non-β regions of APP with antibodies to the C-terminus and N-terminus of APP recognized by some antibodies.

Using tissue fractions & cDNA-transfected cells, we previously showed that sAPP occurs in mammalian tissues as a group of -110-135 kD membrane-associated proteins. Since a -40-residue hydrophobic fragment of sAPP forms the SAP (AA) deposits in AD & these occur outside cells, the processing of sAPP into such fragments should be elucidated. In both human brain & transfected cells, sAPP C-terminal antisera detect all 135 kD membrane-associated proteins that appear to be a favored & stable proteolytic fragment containing the C-terminus & presumably SAP. SAP+g-transfected cells produce more 11 kD soluble sAPP forms in human plasma that comigrate with soluble forms previously described in CSF. These appear to lack the C-terminus, suggesting that the large N-terminal portion of sAPP is released into extracellular fluids following cleavage near the transmembrane region. The relevance of these various forms to s-amyloidosis will be discussed.

ALZHEIMER'S DISEASE DOES NOT ALTER THE PROPORTION OF HIPPOCAPAL NEURONS WITH APP-695 OR APP-751 mRNA. S.A. Johnson and C.E. Finch, Andrus Gerontology Center, Univ. Southern California, Los Angeles, CA 90089-0191.

Northern blot analyses have shown a selective decrease of the amyloid precursor protein mRNA which lacks a Kunitz protease inhibitor motif (APP-695 mRNA) in Alzheimer's disease (AD) cortex and hippocampus. This selective APP-695 mRNA reduction could be due to: 1) a loss of neurons which only express APP-695 mRNA; 2) decreased stability of APP-695 mRNA (or increased stability of APP-751 mRNA); 3) an alteration of de novo transcription of APP-751 mRNA; or 4) an alteration of stability of APP-695 mRNA (or increased stability of APP-751 mRNA). We have addressed the first alternative by high criterion in situ hybridization of AD and control hippocampus with APP-695 and APP-751 mRNA specific cDNA probes. Serial sections were hybridized separately to each probe. Every nucleolated pyramidal neuron with a signal above local background levels was counted throughout the Cornu Ammonis subfields of 7 AD and 5 control brains. There are equal numbers of APP-695 and APP-751 transcript specific neurons in AD and control specimens. This analysis suggests that a specific loss of neurons which only express APP-695 mRNA does not occur in AD hippocampus, and is not a mechanism to explain the selective reduction of APP-695 mRNA.

These studies were supported by grants to CEF (AG07909) and S.A., Investigator Initiated Research Grant.

PROTEIN KINASE CHANGES IN AGED ALUMINUM-TREATED RATS. M.P. Bruyns*, and A. F. Salama (SPON: R.D. Krell). Department of Physiology, Medical College of Virginia, Richmond, VA 23298-0551.

Four protein kinases that copurify with neurofilaments (NF) and microtubules (MT) were assayed in brain and spinal cords of young (1 mo) and aged (26 mo) rats. The activities of 3 of these kinases were substantially decreased in aged rat tissue. These 3 were CAMP-dependent kinase and a cofactor-independent (NI) kinase associated with NF and a cofactor-independent MT kinase. Al3[SO4]2 (100uM) was administered to NF (3%) in drinking water for 1 mo to young and aged rats. Another group of aged rats was given 0.3% AI for 1.5 yr (from 1 yr of age). All kinases were significantly elevated in aged rats after Al treatment. After 1 mo and 1.5 yr CAMP kinase was elevated 3.5-4.0 fold, respectively, NI kinase by 67 and 32%, and MT kinase by 15 and 42%, Ca-calmodulin dependent kinase (with NF) was elevated only after 1 mo, by 138%. Al treatment did not affect the ability of NF proteins to incorporate [32P]Pi. The results of these experiments demonstrate that oligofructose may be capable of surviving and developing even when transplanted into old host brain environment. These results suggest that regeneration and nerve cell replacement may be possible in the aging mammalian brain.

SOLUTE DERIVATIVES OF THE ALAMYLOID PROTEIN PRECURSOR: PURIFICATION FROM CEREBROSAL FLUID AND SEQUENCE ANALYSIS. M. P. PALMERT, T. L. ROSENBERRY, AND S. G. YOUNKIN. Inst. of Pathology, Div. of Neuropathology, and Dept. of Pharmacology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

The amyloid deposits in Alzheimer's disease (AD) is composed primarily of a small 40 kD protein (APP) that is derived from the c-amyloid precursor protein (sAPP). In previous studies, we used antisera to synthetic sAPP peptides to identify (1) a set of ~110-135 kDa membrane-associated proteins that represent full-length forms of the sAPP and (2) soluble ~100-125 kDa derivatives of the sAPP that lack the COOH-terminus of the full length, membrane-associated form. In this study, we have confirmed our identification of the soluble sAPP derivatives by copurifying the ~100-125 kDa proteins, from human cerebrospinal fluid (CSF) by ammonium sulfate fractionation, FPLC using a Mono Q column, and preparative SDS-PAGE. The two proteins were then separately excised from the gel, eluted, subjected to SDS-PAGE, transferred to Immobilon, and sequenced. Nineteen of the 20 amino acids at the NH2-terminus of the more abundant ~100 kDa protein were identical to those predicted from the published sAPP cDNA sequence. Eleven of the 13 amino acids at the NH2-terminus of the ~125 kDa protein were also detectable, and all 11 were identical to those predicted. It is possible that the soluble sAPP derivatives identified in this study are generated by cleavage at either the NH2- or COOH-terminus of the sAPP, which is encoded as an internal peptide in the full-length sAPP molecule. Since these proteins can be readily purified from CSF, it should be possible to characterize their COOH-terminus, identify the specific site at which the sAPP is cleaved during their generation, and determine if this site is relevant to sAPP metabolism.

AGING II

PROTEIN KINASE CHANGES IN AGED ALUMINUM-TREATED RATS. M.P. Bruyns*, and A. F. Salama (SPON: R.D. Krell). Department of Physiology, Medical College of Virginia, Richmond, VA 23298-0551.

Four protein kinases that copurify with neurofilaments (NF) and microtubules (MT) were assayed in brain and spinal cords of young (1 mo) and aged (26 mo) rats. The activities of 3 of these kinases were substantially decreased in aged rat tissue. These 3 were CAMP-dependent kinase and a cofactor-independent (NI) kinase associated with NF and a cofactor-independent MT kinase. Al3[SO4]2 (100uM) was administered to NF (3%) in drinking water for 1 mo to young and aged rats. Another group of aged rats was given 0.3% AI for 1.5 yr (from 1 yr of age). All kinases were significantly elevated in aged rats after AI treatment. After 1 mo and 1.5 yr CAMP kinase was elevated 3.5-4.0 fold, respectively, NI kinase by 67 and 32%, and MT kinase by 15 and 42%, Ca-calmodulin dependent kinase (with NF) was elevated only after 1 mo, by 138%. AI treatment did not affect the ability of NF proteins to incorporate [32P]Pi. The results of these experiments demonstrate that oligofructose may be capable of surviving and developing even when transplanted into old host brain environment. These results suggest that regeneration and nerve cell replacement may be possible in the aging mammalian brain.

TRANSPLANTATION AND SURVIVAL OF NEURONS IN THE BRAIN OF OLD HAMSTERS. E. E. Morrison and R. M. Costanzo. Department of Physiology, Medical College of Virginia, Richmond, VA 23298-0551.

In previous studies olfactory neurons have been successfully transplanted into the brain of neonate or young adult host animals. The present study was undertaken to determine if the "old" brain could serve as a site for the transplantation of neurons. Old hamsters (1-2 years) were used as host animals. Olfactory neurons were obtained from the septal region of neonate hamsters and transplanted into frontal or parietal host cortex. For periods up to 115 days transplanted neurons survived and continued to develop in the "old" host brain environment. Mitotic figures were observed among transplanted neurons and developing axons originating from these neurons grew into the host cortex. These results demonstrate that olfactory neurons are capable of surviving and developing even when transplanted into old host brain environment. These results suggest that regeneration and nerve cell replacement may be possible in the aging mammalian brain.
AGE-DEPENDENT RESPONSE OF NEURITE OUTGROWTH FROM GANGLIA OF PHYSIOLOGY, AND CHEM. DEPT., RUTGERS UNIVERSITY, NEWARK, NEW JERSEY, AND CHEM. DEPT., SETON HALL UNIVERSITY, SOUTH NUTLEY, NEW JERSEY.

We have shown that 12-O-tetradecanoylphorbol 13-acetate (TPA) and other activators of protein kinase C (PK-C) can be potent promoters of neurite outgrowth from explanted sensory ganglia of embryonic chicken embryos. This response to TPA was both age- and dose-dependent. At 20 ng/ml TPA was effective only on explants from young embryos (60-120 h) while those from embryos harvested up to 500 h old did not respond to TPA. Explants from embryos beyond 18 d of age did not respond to TPA treatment. This decreased response was not due to decreased levels of PK-C activity. In contrast, explants from older embryos contained higher levels of PK-C activity. In addition, the lack of response was not due to the loss of receptors for TPA since phorbol ester binding was maximal in older ganglia. These results suggest that while neurite outgrowth requires the activation of PK-C, additional factors other than receptor binding and PK-C activity are also needed for this process.

(Supported by NS12621)
RNA was isolated from whole rat brains and hormone systems have been shown to contribute to heat exposure. Young and old animals reaching the same body weight showed no difference in core body temperatures (CBT) after exposure to heat stress. At all conditions young animals showed consistently higher HSP 70 mRNA induction as compared to the aged group. However, in the control group, the aged animals showed a decreased functional response to nicotine observed in the striatum of aged animals, with no statistically significant difference between the two groups. The results indicate that there is a decrease in the functional coupling of G-protein with catalytic unit and the reduced catalytic unit activity.
AGE-RELATED COMPARISONS IN DETOUR MAZE PERFORMANCE USING A LEARNING SET PARADIGM WITH RATS. E.L. Bresnahan1, N. Muth1, A. Abramson1, R. Cutler1, E.L. Spangler1, and D.K. Ingram2.

1Essex Community College, Baltimore, MD 21237. 2Geronotology Research Center, NIA, Baltimore, MD 21224

A shock avoidance maze task provided within subjects analysis of age-related performance differences using a learning set approach. After training to avoid footshock in the straight arm (SA) portion of the maze, male rats aged 6 (16), 11 (26), and 24 (40) months old were trained over 3 to 5 months in a problem-solving task. Three pairs of U-shaped alleys extended to each side of the SA. Clear barriers could be placed in the runway between any detour pair to force the rat to go right or left. One of the pairs was blocked at the end of the SA alley. On forced trials, entrance to one side of a detour pair was closed; on choice trials, entrances to both alleys were open. Problem complexity was manipulated by increasing number of detour pairs required (1, 2, 3, or 6). Six problems were given weekly, 1 problem/day, 14 trials/day (2 sample, 5 choice, repeated twice). A weekly 85% correct choice-trial criterion was required before moving to the next level of complexity. Y and M rats attained criterion within 1 wk on 1-D problems, 2 wk on 2-D, and 1 wk on 3-D. The A rats required extensive training on both 1- and 2-D problems, attaining criterion on 1-D but not on 2-D. On all measures (errors, correct trials, sample and choice runtimes, Y and M rats differed significantly from A rats but not from each other. After a 3-mo interval when M rats were 22-mo old, testing was repeated. At all complexity levels, performance during relearning was significantly more accurate than during acquisition. Runtimes remained unchanged. Because of improved choice accuracy by now-aged rats trained 6 mo earlier, memory function appeared unchanged, which differs from the cross-sectional perspective, perhaps because of previous experience or differences in health between cohorts.

Norepinephrine (NE) Stimulates Adenosine 3',5'- Monophosphate (cAMP) Production in the Olfactory Bulbs (OB) of Aged But Not Young Male Rats. T. Mencio-Wszalek, D.E. Bluenen and V.J. Ingram.

Department of Psychology, Virginia Commonwealth University, Richmond, VA 23284-0001.

In the present experiment, OB from young (less than 100 days old), middle-aged (greater than 100 days old) and old (greater than 100 days old) male rats were used. Samples from homogenized OB tissue extracts were incubated with one of two different dilutions of cAMP were assayed by radioimmunoassay. cAMP production was inhibited to 70% in 1-D problems, 2 wk on 2-D, and 1 wk on 3-D. The A rats required extensive training on both 1- and 2-D problems, attaining criterion on 1-D but not on 2-D. On all measures (errors, correct trials, sample and choice runtimes, Y and M rats differed significantly from A rats but not from each other. After a 3-mo interval when M rats were 22-mo old, testing was repeated. At all complexity levels, performance during relearning was significantly more accurate than during acquisition. Runtimes remained unchanged. Because of improved choice accuracy by now-aged rats trained 6 mo earlier, memory function appeared unchanged, which differs from the cross-sectional perspective, perhaps because of previous experience or differences in health between cohorts.


Neuroendocrinology Lab., Dept. of Neurology and Medicine and Inst. of Gerontology, Univ. of Michigan and VA Med. Center Greccy, Ann Arbor, MI 48105.

Brain regional opioid peptide concentrations and receptor densities change during aging (Abu-Amer, et al., Brain Res 259:173, 1983). To determine the potential functional significance of these changes, we examined cardiovascular and plasma catecholamine responses to a m-selective opioid peptide, [Dala2, met5] enkephalin (DAGO) injected intracerebroventricularly (icv) in 6 (n=11) and 24 (n=7) month old Fisher 344 rats. Guide cannulae were implanted by stereotaxic approach. After training to avoid footshock in the straight arm (SA) portion of the maze, male rats aged 6 (16), 11 (26), and 24 (40) months old were trained over 3 to 5 months in a problem-solving task. Three pairs of U-shaped alleys extended to each side of the SA. Clear barriers could be placed in the runway between any detour pair to force the rat to go right or left. One of the pairs was blocked at the end of the SA alley. On forced trials, entrance to one side of a detour pair was closed; on choice trials, entrances to both alleys were open. Problem complexity was manipulated by increasing number of detour pairs required (1, 2, 3, or 6). Six problems were given weekly, 1 problem/day, 14 trials/day (2 sample, 5 choice, repeated twice). A weekly 85% correct choice-trial criterion was required before moving to the next level of complexity. Y and M rats attained criterion within 1 wk on 1-D problems, 2 wk on 2-D, and 1 wk on 3-D. The A rats required extensive training on both 1- and 2-D problems, attaining criterion on 1-D but not on 2-D. On all measures (errors, correct trials, sample and choice runtimes, Y and M rats differed significantly from A rats but not from each other. After a 3-mo interval when M rats were 22-mo old, testing was repeated. At all complexity levels, performance during relearning was significantly more accurate than during acquisition. Runtimes remained unchanged. Because of improved choice accuracy by now-aged rats trained 6 mo earlier, memory function appeared unchanged, which differs from the cross-sectional perspective, perhaps because of previous experience or differences in health between cohorts.

The clA effect of NE on cAMP production in brain tissue. These results suggest an interesting dichotomy in the effect of NE on cAMP production in olfactory tissue from rats of different ages. After work has shown that 10^7 M NE is capable of stimulating cAMP production in brain tissue. These results suggest an interesting dichotomy in the effect of NE on cAMP production in olfactory tissue from rats of different ages.

Without experimental intervention, degenerative lesions similar to neuritic plaques have been found in the olfactory cortical and subcortical regions of aged rats. These changes may be initiated from insult to the NOO, particularly the olfactory mucosa, by outside infectious agents or chronic exposure to environmental toxins, secondarily affecting olfactory nerves. The mitral cell glomerular layer exhibited a loss of synaptic density and a decrease in the size of the remaining synapses.

Supported by BRSG #6-32724


Many processes associated with calcium homeostasis change with aging. We have examined the effects of aging on the ability of inositol 1,4,5-trisphosphate (IP3) to mobilize calcium from microsomes prepared from cerebral cortex, hippocampus, thalamus, and cerebellum of 3, 16, and 28-month-old Fischer 344 rats. Microsomal calcium uptake was first stimulated by ATP and then inhibited with sodium orthovanadate. No aged-related differences were found in the ability of brain microsomes to sequester calcium in response to ATP stimulation, but an inter-regional difference was seen. The average calcium-sequestrating capacity in cerebellum was 3.4 nmol/mg protein, followed by cerebral cortex (2.6), hippocampus (2.1), and thalamus (1.4).

A maximally effective dose of IP3 (1 µM) released approximately 30% of the calcium sequestered by microsomes in all brain areas and age groups studied except in the cerebral cortex where a significant effect of aging was observed. In cortex, 1 µM IP3 released 0.77±0.04, 0.81±0.04, and 0.40±0.02 nmol calcium/mg protein in 3, 16, and 28 months of age, respectively; corresponding to responses of 30%, 30%, and 15%. Dose-response curves for IP3 confirmed that neither the maximally effective doses (1 and 1 µM), the EC50 values (133 and 138 nM) nor the Hill coefficients (0.91 and 1.1) differed in cortex from 3 and 28-month-old rats. These data indicate that the efficacy of IP3 is selectively diminished in the cerebral cortex of aged rats. Supported by USPHS AG04418 and the PMA Foundation.


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542.24 ACOUS T AND LONG-TERM CALORIC RESTRICTION EFFECT COORDINATED MOTOR PERFORMANCE OF THREE MOUSE GENOTYPES. M. Forster, M. Flores* and H. Laal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2650.

Caloric restriction regimens which increase longevity of rodents have been shown to retard age-associated changes in CNS processes related to sensorimotor functions. The current studies investigated the effects of long-term caloric restriction on sensorimotor functions in the context of different genotypes and acute diet conditions. Chronically diet-restricted or ad lib fed mice of three genotypes (C57BL/6N, DBA2/N and B6D2Fi) aged approximately 8, 17, or 26 months were obtained from the NCTR-NIA Project on Caloric Restriction colonies.

Half of the mice in each diet condition were switched and maintained under the opposite condition for four weeks prior to testing in迷路 behavioral and physiological tests. Restricted mice were switched to a night-feeding regimen to synchronize their circadian metabolic and behavioral variations with the ad lib mice. Age-related declines in performance capacity for a rotating-treadmill task were delayed in C57BL/6N and B6D2Fi genotypes following long-term caloric restriction, regardless of the acute restriction condition. Acute restriction had a marked effect upon treadmill performance at each age in all genotypes, suggesting that evaluations of the effects of caloric restriction upon age-related behavioral changes will require careful consideration of the role of short-term factors associated with experimental diet conditions. [Supported by NIH grants AG07695 (H.L.) and AG06182 (M.J.F.)]


Possible alterations in muscarinic cholinergic (mch) signal transduction (Snd) in senescence were studied in neostriatum (NST). Activation of m receptors by carbachol or oxotremore inhi bit dopamine DA autoreceptors elicits 4'-phosphate (cAMP) and/or G-protein (Gp) coupled to cAMP accumulation and calcium mobilization (CaM) from NST of perfused brains of 3 or 24 month old rats. However, age deficits in CaM are not seen if the CaM agonists, 8-Br-cAMP or 4'-methyl cAMP, are applied. Present experiments determined effects of simultaneous activation or inhibition/inhibition of more than one second messenger system, IP3, on cAMP accumulation.

Results indicate that these systems interact with each other, suggesting that the mechanisms underlying age-related deficits in the NST include changes in the ability of the second messenger systems to activate the synthesis of the first messenger system. In addition, these results support the hypothesis that the expression of age-related deficits in the NST is associated with changes in the ability of the second messenger systems to interact with each other.

542.26 AGE RELATED CHANGES IN FAST AXONAL TRANSPORT: RETROGRADE FEEDBACK MAY REGULATE ANTEROGRADE SPEEDS. A.C. Breuer and M.B. Atkins*. Neurology and Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44195.

The hypothesis that fast axonal transport (FAT) may be involved in age-related changes in nerve and muscle has been evaluated using indirect techniques (ligand-accumulation and radio-histochemical analyses) with variable results. We report direct observations on the FAT of organelles in individual sciatic axons in newborn, 1,2,3 week old, 1,4, and 8 month old Sprague-Dawley rats using computerized image analog and digital enhanced interference contrast images recorded in real time. Mean retrograde (RFT) FAT speeds were 1.5±0.02 µm/sec and mean anterograde (FA) FAT speeds were 1.4±0.06 µm/sec at birth. By the end of the first month of development mean RFT speed had increased significantly to 1.74±0.05 µm/sec (p<0.001) while mean ANTERO speed had decreased to 1.33±0.06 µm/sec (NS). Thereafter, there appears to be a convergent trend of FAT speeds at 8 months (ANTEC = 1.46±0.03 and RFTO = 1.48±0.02). We interpret these findings as reflecting the regulation of material delivery per unit time, that during early rapid growth, diminished RETRO feedback to the cell soma signals a high utilization rate of organelles/membrane and need for high ANTERO supply. As the animal matures and greater turn around (RETRO) recycling of membrane to the cell soma occurs, indicating less consumption, lower ANTERO supply is required.
EXOGENOUS MOUSE NERVE GROWTH FACTOR STIMULATES CHOLINE ACETYLTRANSFERASE ACTIVITY IN AGED MALE FISHER 344 RATS. J. L. Pyler and L. R. Donald. CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Sensitization of basal forebrain cholinergic neurons to injection of NGF is an NGF-sensitive process that occurs in young and old (2 years old) rats. Normal 2 year old (Aged) male Fisher 344 rats had significant losses of ChAT activity compared to normal 4 month old (Young) rats in micro-dissections of the septum and diagonal band (MS/DB), and caudate-putamen (C-P), as well as the frontal, temporal, and hippocampal cortices. ChAT activity in the Young MS/DB was 2.4 pmol ACh/µg protein/min (n=10) compared to 1.9 (n=21) in the Aged MS/DB (p<0.05). Continuous infusion of NGF (1.2 µg/day for 2 weeks) into the right lateral ventricle had little effect on ChAT activity in the basal forebrain of young rats, as in the previous report. However, NGF treatment induced a 150% supranormal stimulation of ChAT activity in the MS/DB and C-P (n=6, p<0.01) of Aged rats. A significant stimulation of ChAT was also detected in the basal nucleus of Meynert and cortices of NGF-treated Aged rats. This age-dependent sensitization to exogenous NGF may underlie the behavioral improvement and morphological effects found in aged animals after NGF treatment (Nature329:65).


Nerve growth factor (NGF) plays a role in the survival and transmitter function of cholinergic neurons in the CNS. NGF can prevent retrograde degeneration of rat basal forebrain cholinergic neurons following axotomy (PNAS 83: 9231), and decreased levels of NGF may be related to the dysfunction associated with Alzheimer’s Disease. In the present study, NGF treatment for 2 weeks to adult (4 month) and aged (24 month) Fisher 344 rats using an Alzet minipump Control rats received a vehicle infusion or were untreated. Choline acetyltransferase (ChAT) and high-affinity choline uptake (HACU) activity were measured in striatum, frontal cortex, and hippocampus. In NGF-treated aged rats, HACU was significantly increased by 57% and 39% in striatum and frontal cortex, respectively, while this measure was not altered significantly in hippocampus; ChAT activity was increased by 72%, 28%, and 23% in striatum, cortex and hippocampus. In NGF-treated adult rats, HACU was increased by 86% in striatum, but not significantly changed in frontal cortex or hippocampus; ChAT activity was stimulated only in striatum. The results demonstrate for the first time that exogenous NGF can stimulate HACU in vivo, and indicate a differential sensitization of cholinergic markers in aged rats.
REDUCED NUMBERS OF HYPOTHALAMIC BETA-ENDORPHIN
(B-END) AND LHUR NEURONS IN AGED FEMALES
HIV-1 INFECTION OF HUMAN Fetal NERVOUS SYSTEM
ORGANOTYPIC TO serotyping of human CNS cultures. Because this
results in persistent infection in which levels of infectious virus
and viral antigen fluctuate in a cyclical manner. To
determine if hybridized tissue sections for absence of inflammation and/or
inflammation (0.0 ± 0.0). Both groups of mice mounted
an immune response to DAV as indicated by presence of
antibody to DAV as measured by ELISA assay against
purified DAV antigen. This variant will be useful to study
the viral determinants important in virus-induced
demyelination. (Supported by NHI grant NS 24180, Multiple
Sclerosis grant RO-1878, and Searle Scholar Award).

TRELLER'S VIRUS ISOLATED FROM A PERSISTENTLY INFECTED
OLIGODENDROGLIOMA CELL LINE FAILS TO INDUCE DEMYELINATING
DISEASE IN CENTRAL NERVOUS SYSTEM OF SJL/J MICE.
A. K. Patrick* and N. Rodriguez* (SPON: V. Lennon) Dept. of
Immunology, Mayo Clinic, Rochester, MN 55905.
Infection of the G26-20 oligodendrogliona cell line with the Da strain of Thaller's virus (DAV) results in persistent infection in which levels of infectious virus
and viral antigen fluctuate in a cyclical manner. To
determine if hybridized tissue sections for absence of inflammation and/or
demyelination (p<0.05), and 25% fewer LHUR neurons (p<0.0001) in
the region of the preoptic area and the diagonal band of Broca compared to young mice. No change in distribution was observed for either type of neuron. These results indicate that B-End-containing neurons partly account for the 30% decreases in anti-opio-
CNS cultures 

HIV-1 INFECTION OF HUMAN Fetal NERVOUS SYSTEM ORGANOTYPIC
Previous studies from our laboratories have shown that HIV-1 can directly infect organotypic cultures of human fetal central nervous system (CNS) tissue. Because this
may not represent the method by which HIV-1 enters and
affects the CNS in vivo. Studies were conducted to
investigate the ability of HIV-1 to infect fetal lymphocytes and for these cells to act as a vector for HIV-1 infection. foci were scored from tissue culture flasks. A combination of rat tail collagen and poly-L-
lysine treatment yielded the highest plating efficiency (70% ± 5). Under
optimal conditions in the logarithmic phase of culture growth, HFA have a
doubling time of 30 hours (± 6). Cell growth occurs as multilayered foci even
under suboptimal conditions (GFAP)-positive astrocytes were established from abortuses of 8-18 weeks
maturation and to define growth requirements and characteristics of
human fetal astroglial (HFA) cell strains with human
immunodeficiency virus (HIV-1). J.J. Chao and W.P. Pantos*
Dept. of Micro. & Pediatrics, Univ. of Miami Sch. of Med., Miami, FL 33136.
Human cell strains consisting predominantly of glial fibrillary acidic protein
(GFAP)-positive astrocytes were established from abortuses of 8-18 weeks
gestation. Several media and substrates were evaluated to determine optimal plating efficiency and growth. Media were supplemented with 5% horse serum; NS, a medium free of selected amino acids and Dulbecco's modified
Eagle's medium were comparable as measured by growth rates whereas RPMI
1640 supported only limited growth. Plating efficiency was evaluated using a
rat tail collagen preparation, commercial collagens, and poly-L-lysine to
pretreat tissue culture flasks. A combination of rat tail collagen and poly-L-
lysine treatment yielded the highest plating efficiency (70% ± 5). Under
optimal conditions in the logarithmic phase of culture growth, HFA have a
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immunodeficiency virus (HIV-1). J.J. Chao and W.P. Pantos*
Dept. of Micro. & Pediatrics, Univ. of Miami Sch. of Med., Miami, FL 33136.
we have investigated HSV infection in sensory neuronal cultures after treatment with phorbol ester. This suggests that at least two prevented the reactivation of latent HSV after NGF deprivation and elevate cyclic AMP, and that activation of protein kinase C with pharmacological agents. Recently we found that agents which and found that latent HSV infection was also reactivated after NGF phorbol ester produced reactivation of latent virus. An agent, 2-

and that deprivation of nerve growth factor (NGF) results in Denver, CO 80262

neural crest-derived sympathetic and sensory neurons. The NEURONS HSV infections are established in sympathetic neuronal cultures 1

S 1 Pediatrics, University of Colorado Health Sciences Center,  

J. Neurochem. 51: 1946, 1988). To investigate QUIN and L-TRP metabolism and the relationship of QUIN to AIDS dementia complex (ADC), QUIN and L-TRP were quantified in CSF and plasma of 20 HIV-infected patients. Plasma L-kynurenine (L-KYN) was measured in 9 control and 13 AIDS patients. The results (Table) showed markedly increased QUIN and reduced L-TRP in CSF and plasma of HIV-infected patients, particularly those with ADC and opportunistic conditions. Plasma L-kynurenine concentrations were also increased in AIDS.

The results are consistent with increased L-TRP metabolism through the kynurenine pathway and may reflect activation of indoleamine-2,3-dioxygenase. In demented patients without underlying illness, significant correlations were found between log CSF QUIN and AIDS dementia complex stage (p<0.001), CSF beta2 (p<0.005), gamma globulin (p<0.001), and cerebrospinal fluid (CSF) QUIN and L-TRP concentrations. Plasma L-kynurenine concentrations were also increased in AIDS patients.

HIV-1 expression in neural cells is dependent on non CD4 associated entry and cellular permissiveness to virus expression (J. Neurosci. Res. 19: 705-711, 1988). We have shown that neonatal infection with an attenuated HSV-1 produces hyperactivity in mice (Crnic & Pizer, Neurotoxicology. 11: 109-118, 1988). Now does the virus enter the CNS? Newborn mice were injected subcutaneously, in the shoulder with a low virulence mutant strain of HSV-1 which lacked Thymidine kinase expression. At 72 hr post infection no organism could be identified as a presumptive source of infection, the delay to begin infection being 13 months. To the organism isolated included Propionibacterium in 3 patients, 2 of which had mixed Propionibacterium and Staphylococcus epidermidis infections, alpha-hemolytic Streptococcus in 1, and in another patient no organism could be identified. The specter of shunt infection occurring years or decades following shunt insertion is common within the first weeks following shunt insertion due to organisms implanted at surgery. We have observed unusual delayed infections occurring years or decades after insertion. Cases of shunt infection at our institution were reviewed from 1979 to 1987. Twelve cases were identified where infections occurred more than 6 months following shunt insertion, in children aged 6 mo to 17 yr. Seven had recent surgery or infections that immediately preceded shunt infection. For five patients, no antecedent infection or infection could be identified as a presumptive source of infection, the delay to begin infection being 13 months. The organisms isolated included Propionibacterium in 3 patients, 2 of which had mixed Propionibacterium and Staphylococcus epidermidis infections, alpha-hemolytic Streptococcus in 1, and in another patient no organism could be identified. The specter of shunt infection occurring years or decades following shunt insertion is common within the first weeks following shunt insertion due to organisms implanted at surgery. We have observed unusual delayed infections occurring years or decades after insertion. Cases of shunt infection at our institution were reviewed from 1979 to 1987. Twelve cases were identified where infections occurred more than 6 months following shunt insertion, in children aged 6 mo to 17 yr. Seven had recent surgery or infections that immediately preceded shunt infection. For five patients, no antecedent infection or infection could be identified as a presumptive source of infection, the delay to begin infection being 13 months.

To examine the extent and dynamics of its turnover, the sizes and frequencies of an identified class of synaptic contacts formed by terminals of photoreceptors were characterized using quantitative EM methods. The influence of visual experience upon these parameters was examined in the lamina (the first optic neuropile, or lamina, of the fly Musca, where it was seen most often in the choroid plexus). In contrast, MAb 3G8 reactivity (FcRIII) was only seen in the subependymal periventricular tissues. These results demonstrate that regions of adult brain which produce CSF and border those seen in CSF from patients with chronic viral infections.


The giant fiber (GF) pathway of Drosophila melanogaster mediates the stereotyped escape response of the adult. The GF drives, via electrical synapses, the jump muscle motoneuron (TMN) which activates the jump muscle (TM) and the interneuron, PSI, which chemically synapses onto the TMN. MAb 3G8 reactivity (FcRIII) was only seen in the subependymal periventricular tissues. These results demonstrate that regions of adult brain which produce CSF and border regions of adult brain which produce CSF and border

SYNAPTONECROSIS II


The giant fiber (GF) pathway of Drosophila melanogaster mediates the stereotyped escape response of the adult. The GF drives, via electrical synapses, the jump muscle motoneuron (TMN) which activates the jump muscle (TM) and the interneuron, PSI, which chemically synapses onto the TMN. MAb 3G8 reactivity (FcRIII) was only seen in the subependymal periventricular tissues. These results demonstrate that regions of adult brain which produce CSF and border regions of adult brain which produce CSF and border


To examine the extent and dynamics of its turnover, the sizes and frequencies of an identified class of synaptic contacts formed by terminals of photoreceptors were characterized using quantitative EM methods. The influence of visual experience upon these parameters was examined in the lamina (the first optic neuropile, or lamina, of the fly Musca, where it was seen most often in the choroid plexus). In contrast, MAb 3G8 reactivity (FcRIII) was only seen in the subependymal periventricular tissues. These results demonstrate that regions of adult brain which produce CSF and border

A recent technique for photo-degeneration of receptor cells in the compound eye (Picaud, S. et al., Neurosci. Lett., 95:24, 1988) offers the opportunity to examine, after synaptogenesis, we looked for the presence of antero- synaptic ribbons [or of postsynaptic sites] decline < 37%[22%] in density. G a p  j u n c tio n s  are s till p rese n t b e tw e e n  n eu ro n s and presynaptic ribbon in the degenerating terminal is lost; 2) the processes of a glial cell insinuate between pre- and postsynaptic membranes; and 4) the components of the ensemble then separate. 3) the processes of a glial cell insinuate between pre- and postsynaptic membranes; and 4) the components of the ensemble then separate. As a result, the frequencies of presynaptic ribbon formation decrease. The absence of a glial cell process from the postsynaptic site, as in some cases, suggests that axon terminals may contribute to the formation of postsynaptic ribbons. The data indicate that in the presence of anterogradely labelled terminals, the presence of a glial cell process is necessary for the formation of postsynaptic ribbons. The absence of a glial cell process from the postsynaptic site, as in some cases, suggests that axon terminals may contribute to the formation of postsynaptic ribbons. The data indicate that in the presence of anterogradely labelled terminals, the presence of a glial cell process is necessary for the formation of postsynaptic ribbons.


test results that do not show changes and maybe considered uninteresting. Synapses first appear on day 3 or 4 in culture, and their number increases rapidly thereafter. Synapses were never observed before day 3, even though numerous contacts between axons and other cells developed within the first 3 days. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target. The delay in the appearance of presynaptic specializations could be related to the maturation of the presynaptic axon or the postsynaptic target.
ULTRASTRUCTURE OF THE DEVELOPING CHICK TANGENTIAL VESTIBULAR NUCLEUS FOLLOWING OCOTOCYST ABLATION: R. Fritzsch, P.G.H. Clarke* and B.O. Grinnell. Dept. of Anatomy, Univ. of Virginia, Charlottesville.  VA 22908. We have studied the development of fibers reaching the inner tangential nucleus (TN) in the chick vestibular system following early otocyst ablation, which prevents the ingrowth of primary vestibular afferents. TN neurons still migrate and differentiate normally up to 8 days (Golgi studies: Peusner and Mostert 1977). Ultrastructural studies of normal embryos from 5 to 8 days have shown that synapses in the TN are formed mainly by longitudinal fibers of unknown origins on the processes of primitive epithelial cells (PEC). PEC are neuron precursors.

In the present ultrastructural study of otocyst-ablated embryos, we have determined that synapses form at the normal times (between 5 and 8 days) and between the normal synaptic partners. Therefore, migration and differentiation of TN neurons may depend in part on synapse formation by the longitudinal fibers, some of which must be of non-vestibular origins. Supported by NIH grant RO1 NS18108.

544.11 TIME OF ORIGIN OF TARGET-SPECIFIC SYMPATHETIC GANGLION NEURONS. L.L. Wright, A.F. Elshtain and C. Skeaton, Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118. Much of what we know about postganglionic sympathetic neurons is based on studies of whole ganglia. However, neurons of the rat superior cervical ganglion (SCG) innervate a wide variety of tissues, including the submandibular gland and iris, and are therefore potentially functionally heterogeneous. To determine whether neurons born at different times selectively innervate different target tissues, pregnant rats were injected with tritiated thymidine on gestation day (G)15, 18, or 20 to label neurons of the somatic and parasympathetic nervous systems. At G18 and in hatched chicks the restricted type terminates on tanned-shaped cells in the amacrine sublayer, whereas the widespread type remains in lamina 1 of the IPL, spreading tangentially for more than 1mm. The differences in size and pattern reported here are much more pronounced than in most previous reports, but match the description of target fibers. We have evidence that the restricted type originates from the tangential nucleus of the chick nervus opticus. Supported in part by NIH grant NS20494.

544.12 ACQUISITION OF SYNAPTIC PROPERTIES BY THE GROWTH CONES OF SEROTONERGIC AXONS IN DEVELOPING RAT BRAIN. N. Yuj, M.D. H. Tamati and M.D. Gerasch. (SPON: L. Role) Dept. Anat. and Cell Biol. Columbia Univ. P&S, New York, NY. 10032. Stages in the remodelling of the growth cones of serotonergic axons into neurons were investigated. Procedures included the acquisition of synaptic vesicles and the plasma membrane 5-HT transporter. We have previously reported that 5-HT is present and that high affinity 5-HT binding sites are significant in growth cones isolated at day E15 from developing rat brain. We now report that isolated growth cones (IGC) take up 5-HT-5-HT when incubated with that amine (0.5μM). This uptake is inhibited by fluoxetine (20μM) and is temperature dependent. The specific uptake of 5-HT-5-HT develops prior to E20; therefore the 5-HT-imprinting sites in IGC are probably associated with the 5-HT transporter. The ability of reserpine to deplete the 5-HT and the presence of serotonin binding protein (SBP) were used as a markers for the presence of 5-HT-storing synaptic vesicles. Treatment of dams with reserpine (5 mg/kg) does not affect the 5-HT concentration in IGC at E15, but depletes 5-HT from IGC at E20. Administration of reserpine to postnatal animals also depletes 5-HT from IGC. Immunoblot showed that 45 kDa SBP is present and enriched in IGC at E20. These observations support the hypothesis that the growth cones of serotonergic axons acquire the plasma membrane 5-HT transporter before they contain synaptic vesicles; however, synaptic vesicles appear in 5-HT-containing growth cones and thus are present prior to the formation of synapses. The early development of synaptic mechanisms in growth cones is consistent with the possibility that release of 5-HT from growing axons may play a role in early development, even before serotonergic synapses are formed. Supported by grants M H 37575 , NS 15547, and NS 07062.
EFFECTS OF DIFFERENTIALLY-TIMED INJECTIONS OF METHYLISOXYPHTHALIC ACID (MIA) ON THE ANATOMY AND NEUROCHEMISTRY OF THE HIPPOCAMPUS AND THE HABENULO-INTERPEDUNCULAR SYSTEM. A. Conteastabile*, A. Virgili* and O. Barnabei* (SPON: Euro-PEPS) from N I H (MLB and MCW), P M A Foundation (J W P), and March of Dimes (GAO).

The effects of MIA injections on the anatomy and neurochemistry of the developing hippocampus and the habenulo-interpeduncular system (HIS) were studied. MIA injections were given to rat pups at different times during development, from E15 to PN D 5. Nissl staining and electron microscopy were used to analyze the effects of MIA on the development of the hippocampus and the HIS. The results showed that MIA injections at E15 resulted in a decrease in the number of pyramidal neurons and a decrease in the number of synapses within the molecular layer of the hippocampus. These results suggest that developmental synaptogenesis is dependent on MIA receptor activation.

NMDA ACTIVATION AND SYNAPTIC PLASTICITY. T.L. Petit*, W.J. Brooks*, R. Lo*, and J.C. LeBoutillier*. Dept. of Psychology, Univ. of Toronto, Ontario, Canada M1C 1A4

Learning and memory are thought to depend on synaptic plasticity induced by NMDA receptor activation. This paper presents evidence that NMDA activation is a model of learning and memory. If NMDA mediates synaptogenesis, NMDA administration during development should induce increased synaptic plasticity but sensitivity to NMDA should decline with age. Rat pups of different developmental ages were given intraperitoneal injections of NMDA. The results showed that NMDA administration at E15 resulted in a rapid increase in synaptic plasticity, while NMDA administration at PN D 5 resulted in a slower increase. This ability of NMDA to rapidly increase synaptic plasticity is lost over the course of development.


SNAP-25 (Synaptosomal Associated Protein, 25 KDa) is a developmentally regulated protein present in presynaptic terminals of specific regions in mammalian and avian CNS. Expression and localization of SNAP-25 mRNA and protein during development of rat CNS was explored using Western blotting. In situ hybridization, immunoblotting, and immunocytochemistry. Both mRNA and protein levels increased significantly from E15 to adult as detected by Northern and Western blotting. SNAP-25 peptides (25 and 27 KDa) were detected in synaptosomes; the 27 KDa peptide was absent by PND 5. Immunocytochemistry revealed that the SNAP-25 protein was enriched in axons of rat hippocampus and corticospinal tracts during PND 1-14. In adult animals, immunoreactivity was confined to presynaptic terminals of mossy fibers, lateral septal nuclei, and neocortex, but was absent from hippocampal and corticospinal fibers. Subcellular fractionation indicated that SNAP-25 was tightly associated with synaptosomal membranes; the protein was not removed by 1 M NaCl, but was solubilized by 1% Triton X-100. The differential localization of SNAP-25 in axons during development and presynaptic terminals in adult brain may suggest a role for SNAP-25 in plasticity of the developing axon and adult synapse. Supported by grants from NIH (MBL and MCW), PMA Foundation (JWP), and March of Dimes (GAO).
CONDUCTION VELOCITIES OF RETINOFLGAL FIBERS INCREASE BETWEEN OPTIC NERVE AND TRACT IN FERRETS

The largest retinal ganglion axons in the ferret increase in caliber as they course centrally from optic nerve to tract (see Neurosci. Abstr. 14: 992, 1988). Since axon diameter is proportional to conduction velocity, we were able to confirm and extend the finding by measuring the conduction velocities of the two largest classes of retinofugal fibers from records of the compound action potential.

Conduction times for the shortest latency negative peak (t1), thought to represent the axons of Y or retinal ganglion cells, and for the slower peak (t2), thought to represent X or geniculate axons, were measured in 5 adult sable ferrets. Electrodes were positioned near the optic disc, in the optic nerve just behind the eye, in the prechiasmatic nerve, in the optic chiasm, and in the pregeniculate part of the optic tract. Conduction distances were measured directly with silk thread or computed from reconstructed serial sections.

Conduction velocities for the t2 peak in the tract (40 ± 6 m/s mean ± s.e.m.) were consistently much greater than those in the nerve (20 ± 2.4). For the t1 peak, the difference was smaller and more variable (16 ± 3.4, nerve 13 ± 3.6), but the measured tract velocities were greater than or equal to those in the nerve in every case.

These measurements indicate that conduction velocities of both Y and X cells increase along their central course. These changes in axonal diameter and velocity may be related to differences between the glial environments of the optic nerve and tract.

Supported by the Medical Research Council of the U.K.; MPS supported by the N.I.H. and the McKnight Foundation.

THE DISTRIBUTION OF ISOLATERALLY PROJECTING AXONS IN THE CAT OPTIC NERVE AND THEIR RELATIONSHIP TO PATTERNS OF FASCICULATION. L. Jeffery Morgan.

In order to determine the course taken by axons of known retinal origin through the optic nerve in the cat, isolaterally projecting axons have been traced following unilateral injections of HRP into the dLGN. The topographic distribution of these axons changes along the length of the nerve. In the distal (behind the eye) two thirds of the nerve the majority of the labelled axons are confined to a retinotopically appropriate location, that is, linear. However, many labelled axons are also found in central and medial regions. In the proximal third of the nerve an increasing proportion of these axons occupy the central and medial regions, so that in the most proximal third the nerve enters the chiasm isolaterally projecting axons are found over a wide mediolateral extent. Although proximally more axons are located laterally than medially, their general distribution is relatively loose compared with that nearer the eye. The changes in the distribution of these axons along the length of the nerve are gradual.

The change in the distribution of the isolaterally projecting axons is in part related to a change in the organisation of the optic nerve from a fasciculating pattern distally to a relatively nonfascicular pattern proximally. This change takes place gradually close to the chiasm in approximately the proximal one fifth of the nerve.

These results strongly suggest that partial retinotopic order is only present in distal regions of the cat's nerve and that the absence of retinotopic order proximally may be related to a reduction in fasciculation.

(Supported by MRC grant, no. PG 8324037)

MAINTENANCE OF RETINAL PROJECTIONS TO CAT DORSAL LATERAL GENICULATE NUCLEUS FOLLOWING REMOVAL OF POSTSYNAPTIC TARGET CELLS IN THE ADULT.

H. E. Pearson, W. J. Stobart and D. J. Shagass*

To investigate the effects of target cell removal on the survival and maintenance ofafferent, kainic acid (3 nmoi/ml) was injected bilaterally at multiple sites within the dLGN of adult cats. Following post-operative survivals of 2.4 and 6 months, the cats were injected intracranially with HRP. After a further 72 hr, the cats were sacrificed and adjacent brain sections were processed for thionin staining and HRP histochemistry. Thionin stained large regions of dLGN to the dLGN, as characterized by the absence of neurons and increased numbers of glia. Other regions of dLGN were not degenerated and contained normal cells. Sections reacted for HRP showed terminal and preterminal labelling to be present both in regions of normal cells and in regions of degeneration. HRP label was present within these regions of degeneration in all animals studied, regardless of the length of the survival period after kainic acid injection. Nuclei which contained normal and degenerated regions within the same section allowed comparison of the labelling of the two. All nuclei showed evidence for decreased density of label within the degenerated regions, whereas others did not. We conclude that retinal ganglion cells in the adult cat have functional axonal terminals up to 6 months after destruction of their postsynaptic target cells by kainic acid, and may show some capacity for rearrangement of their axonal terminals.

Supported by NS25196.

The retinal terminal types (R1, R2, R3) present in the LGDB are polymorphic. These include terminals with large, dense core vesicles (LCV), medium-sized, dense-core vesicles (MDS), and small, clear vesicles (SCV). The proportion of each type varies depending on the retinal region and the LGDB area.

For low pH, TMB-reacted tissue. Of these, more than 60% are larger than 13 µm and less than 10% are smaller than 10 µm. In contrast, somal sizes for labeled cells in the LGDB injection, labeled cells vary in somal size from 8 µm to 16 µm.

Less than 10% of the labeled cells are larger than 13 µm and less than 10% are smaller than 10 µm. Although the predominant retinal input to the ipsilateral LGDB originates in the larger ganglion cells. [Supported by NIH grants EY05504, EY00126, EY02621.]

545.10

PECTO-THALAMIC PROJECTIONS IN A TYPE 2 LIZARD: TOPOGRAPHY AND CELLS OF ORIGIN. N. M. Montgomery, R. Mergansahl* and K. V. Feite. Univ. Mass./Amherst.

The tecto-thalamic tract of Anolis carolinensis was studied with anterograde and retrograde HRP transport. Using localized tectal injections the degree of topography in the projection was charted. Injections of HRP into n. rotundus and the dorsal and ventral lateral geniculates retrogradely labeled tectal neurons.

Tectal efferents to the pretectal nuclei and to the dorsal and ventral lateral geniculates arise from the superficial and deep tectal layers that are topographically organized. The projection to n. rotundus is not topographic and originates from a single population of neurons in the lower portion of layer 7. The hyperdevelopment of layer 7 is characteristic of Type 2 (iguanaid) lizards. (Northcutt, 1978). Differences in tectal and pretectal cytoarchitecture may be related to the flow of sensory information to cortex. One example is the projection from the dorsal raphe nucleus (DR) to various visual thalamic structures. We studied the anatomical organization of this projection by injecting the DR of cats. Immunohistochemistry demonstrated the presence of a specific population of terminals which contained round vesicles and made asymmetrical synapses. The density of labeled terminal arbors was greatest in the LGNV; intermediate in the LGN¿, the perigeniculate nucleus (PGN) and the visual pulvinar; and very low in the LGN¿m and the visual cortex. Not all of the LGN sites are associated with terminals of efferent afferents, suggesting that there is another population of [Type 2] corticotopic binding sites. Electrophysiological studies were conducted in the LGN of urane anesthetized rats to assess the consequences of activation of nicotine binding sites. Locomotor activity of the rat increased in the spontaneous activity of LGN units. The activity started within 30 seconds and built steadily over the first minute. In addition, the postsynaptic components of field potentials evoked by electrical stimulation of the optic chiasm were increased in size by as much as 50%. With longer ejection durations, however, the unit firing rate gradually decreased and after 5 minutes the cells had ceased responding altogether. Responsiveness to nicotine remained depressed for the next 15-20 minutes, presumably reflecting desensitization of the receptors.

These data demonstrate that functional nicotine receptors are present in the LGN and that their activation increases both spontaneous and evoked activity of LGN units.

545.6

FUNCTIONAL CHARACTERISTICS OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE RAT LATERAL GENICULATE NUCLEUS. G. T. Prusky, M. S. Cynader & R. M. Douglas, Dalhousie Dept. of Psychology, Dalhousie University, Halifax, N.S., B3J 4J1 and Dept. of Ophthalmology, University of British Columbia, Vancouver, B.C., V5Z 3N9, Canada.

The tecto-thalamic tract of Anolis carolinensis was studied with anterograde and retrograde HRP transport. Using localized tectal injections the degree of topography in the projection was charted. Injections of HRP into n. rotundus and the dorsal and ventral lateral geniculates retrogradely labeled tectal neurons. Tectal efferents to the pretectal nuclei and to the dorsal and ventral lateral geniculates arise from the superficial and deep tectal layers that are topographically organized. The projection to n. rotundus is not topographic and originates from a single population of neurons in the lower portion of layer 7. The hyperdevelopment of layer 7 is characteristic of Type 2 (iguanaid) lizards. (Northcutt, 1978). Differences in tectal and pretectal cytoarchitecture may be related to the flow of sensory information to cortex. One example is the projection from the dorsal raphe nucleus (DR) to various visual thalamic structures. We studied the anatomical organization of this projection by injecting the DR of cats. Immunohistochemistry demonstrated the presence of a specific population of terminals which contained round vesicles and made asymmetrical synapses. The density of labeled terminal arbors was greatest in the LGNV; intermediate in the LGN¿, the perigeniculate nucleus (PGN) and the visual pulvinar; and very low in the LGN¿m and the visual cortex. Not all of the LGN sites are associated with terminals of efferent afferents, suggesting that there is another population of [Type 2] corticotopic binding sites. Electrophysiological studies were conducted in the LGN of urane anesthetized rats to assess the consequences of activation of nicotine binding sites. Locomotor activity of the rat increased in the spontaneous activity of LGN units. The activity started within 30 seconds and built steadily over the first minute. In addition, the postsynaptic components of field potentials evoked by electrical stimulation of the optic chiasm were increased in size by as much as 50%. With longer ejection durations, however, the unit firing rate gradually decreased and after 5 minutes the cells had ceased responding altogether. Responsiveness to nicotine remained depressed for the next 15-20 minutes, presumably reflecting desensitization of the receptors.

These data demonstrate that functional nicotine receptors are present in the LGN and that their activation increases both spontaneous and evoked activity of LGN units.
545.11 TECTO-THALAMIC PROJECTIONS IN RANA PIPIENS: TOPOGRAPHY AND CELLS OF ORIGIN. K. V. Fite, H. M. Monteith, and Z. L. Lit. University of Massachusetts/Amherst

The tecto-thalamic tract was studied both with antero- and retrograde HRP transport. Topographic organization of the tract was analyzed following discrete injections of tracer in different zones of the tectum and have different terminations. The most superficial axons are interwoven with optic tract axons, follow a different trajectory to the suprachiasmatic nucleus and posterior lateral nucleus. The most superficial fibers form the tecto-dublar and tecto-spinal tracts, which appear topographic.

545.12 QUANTITATIVE IMMUNOGOLD ANALYSIS REVEALS HIGH GLUTAMATE LEVELS IN AXON COLLATERAL TERMINALS OF GENCULO-CORTICAL RELAY CELLS IN THE PERIGENICULATE NUCLEUS OF THE CAT. S. M. Sherman, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

Synaptic terminals of axon collaterals of geniculo-cortical relay cells in the perigeniculate nucleus (PGN) of the cat have been identified as RLD type (Montero, Exp. Brain Res. 75:5897) characterized by round synaptic vesicles, large size (2-6 μm) and dark mitochondria. In this study we examined the glutamate (Glu) enzyme activity in axon terminals in the PGN, with that of relay cell somatic (RS) and dendrites (RD), interneuron's somatic (IS) and dendrites (ID), retinal (RLP), cortical (RSD) and F terminal in the lateral geniculate nucleus (LGN). In serial thin sections reacted with a GABA antibody IS, ID and RD were GABA- (+) while the other profiles were GABA (-). Mean densities of anti-Glu gold particles per μm² SEM in the different profiles were: RSD 3.0 ± 1.26; RLD 23.78 ± .68; RLP 22.34 ± 84; RD 17.72 ± 78; RS 163.1 ± 47; F 14.19 ± 48; IS 124.1 ± 78; IS 118.1 ± 1.01. Glu IGR differences between RLD and RLP, RD and IS and ID were not significant (p>0.05). The results (a) corroborate in cat LGN similar higher levels of Glu IGR in RSD and RLP terminals than in F terminals previously found in macaque LGN (Montero and Wendt, Neurosc. '89); (b) show 34-46% higher Glu IGR in axon collateral terminals (RLD) than in the parent (RS, RD) geniculo-cortical neurons (p<0.001) suggesting higher levels of Glu associated to the transmitter pool of this amino acid in terminals of geniculo-cortical fibres as neurotransmitters in the geniculo-cortical synapse.


Recently it has been shown that, in the visual sector of the rabbit's thalamic reticular nucleus (TRN), focal regions of visuocortical areas V1 and V2 are represented by components, or "slabs," that lie within the plane of the TRN, run parallel to it's borders, and occupy only a fraction of its thickness. However, in the cat (Sporns et al., Exp. Brain Res. 139; 1989), in the present study of the TRN, injections of HRP and/or [3H]proline were made into visuocortical areas 17 and 18 in the cat and somatosensory cortical area SI in the cat and rabbit. The resultant anterograde labelling in the thalamus was analyzed.

In the cat, 34-46% more than 17% of the total number of fibers tecto-thalamic axons result in single zone of terminal label located within a dorsolateral portion of the TRN; in the cat and rabbit, single injection into area SI results in single zone of terminal label that in part of the TRN lying adjacent to the ventrobasal complex. Each zone lies within the plane of the nucleus parallel to its borders and occupies a small part of its thickness. In the cat and rabbit, injections into the face representation of SI produce labelling within the inner part of the TRN, whilst those into the SI body representation produce labelling within the outer part of the nucleus. Taken together with the previous findings in the rabbit, the present results indicate that the components representing focal regions of cortex within the TRN are similarly organized within both the visual and somatosensory sectors in both the cat and rabbit. Further, in both species there appears to be a topographic map within the TRN's somatosensory sector, one axis of which extends perpendicular to the plane of the nucleus across its thickness. A topographic map within the visual sector of the TRN remains to be determined.

(Supported by the MRC, Grant No. G8707070N)

545.14 A DIRECT PROJECTION FROM THE ANTERIOR INTRALAMINAR NUCLEI TO THE STRIATE CORTEX IN KITTENS. L. Martinez-Hillen*, W. Slisberger and A. L. Perez-Samanin, Dept. of Psychiatry, University of Buenos Aires, 4890 Leoba (Vizcaya), SPAIN.

A direct projection of the anterior intralaminar nuclei to the striate cortex in kittens was shown by retrograde transport of HRP, Fluorogold and Rhodamine beads injected in different parts of inter and extrahemispheric area 17. The projection is ipsilateral and originates in small polymorphic neurons distributed in the three anterior intralaminar nuclei: the central medial, para-central and central lateral.

A projection to the striate cortex presented a unique topographic arrangement as observed when displacement of the labeled area at the injection site in a caudal direction was accompanied by a displacement of labeled intralaminar neurons in a rostral direction.

Complete morphology of rhodamine labeled neurons in slices of paraffinembedded brain and neuropil tissue including the anterior intralaminar complex was seen by intracellular injection of 3-5 µl Lucifer Yellow. According to the distribution of dendrites, two main types of intralaminar projecting cells were observed: multipolar and inverted pyramid-like neurons. Dendritic arborization of these neurons were decorated with thin spines.


We recorded the extracellular responses of 40 X and 75 Y cells in the lateral geniculate nucleus (LGN) of anesthetized cats. Y cells often showed high frequency bursts (we define a burst as 2-5 spikes each separated by 50 ms), while such bursts were rare in X cells. The probability of a burst in a 0.5s standard sampling period during spontaneous firing was larger for Y than X (p<0.001). For Y cells (n=34, p<.001), a 0.5s following a stimulus of the optic chiasm (ECO), this probability dramatically increased for Y cells (83% for Y cells vs. 5% for X cells, p<.001). The typical Y cell burst occurred at a latency of 1.0-1.4ms, however, a 100-200ms silent period precedes a burst discharge, followed by a similar silent period after the burst, etc. Ectopic activation of the parabrachial region (PRB) of the midbrain, which innervates the LGN, did not affect the burst on X cell responses to OK shock. However, PRB activation prior to OK shock in Y cells reduced the burst probability to the level seen with frontal and occipital cortex. Our study of 23 X and 34 Y optic tract axons revealed no such evidence of a burst response, either during spontaneous activity or after OK shock. We thus conclude that this burstiness represents a response pattern during which normal retinogeniculate transmission is blocked, and active PPR input to the LGN can prevent this block.

(Supported by NSF grants EY00308 and EY06082)


From intracellular recording of geniculate X and Y cells in cats, we determined that both cell types have a voltage-dependent, 3-5 mV, hyperpolarization-activated, Ca²⁺-dependent LTS previously described by others for thalamic neurons. The LTS is a triangle wave form, 5-10ms in onset, lasting 40-50ms; is activated at lower thresholds than a standard action potential; usually evokes high frequency bursts of 2-5 action potentials; is inactivated by membrane potentials above roughly -50mV; and typically requires >50ms of membrane hyperpolarization for de-inactivation. EPPs commonly activated LTSs in Y cells, notably after stimulation of the optic nerve. In contrast, the bursts recorded extracellularly are especially prevalent (see Lu et al., this volume). Activation of the midbrain parabrachial region, which effectively blocks the induced bursts in Y cells, reduces LTS frequency in these cells, partly, it seems, by reducing EPSP duration and thus preventing de-inactivation of the LTS. Thus the bursts we recorded extracellularly probably reflect LTSs. EPPs rarely activated LTSs in X cells. Two possible reasons for this difference between X and Y cells are: 1) the LTS was more easily evoked by OK shock, amplitude depolarizes Y cells to activate the LTS, but the X cell EPPs are too small, although larger depolarizations could activate the current injection activate LTSs; 2) Y cells tend to display longer lasting EPPs after OK shock, which may be needed to de-inactivate the LTS.

(Supported by NSF grants EY00308 and EY06082)

Electron microscopic observations on long series of sections of the lateral geniculate nucleus parvocellular and magnocellular laminae in monkeys (M. mulatta) revealed the existence of several triad types. The intercalated element was always a GABA-containing presynaptic dendrite or soma of an internuncial (I-cell), and the output component was invariably a dendrite or soma of a geniculocortical projection or principal neuron (P-cell). The axonal input to the triads, however, could be of three types: (1) the majority were retinal axon terminals; (2) a smaller fraction were the axonal endings of corticofugal fibres that were connected to thin, distal P-cell dendrites; (3) still others were GABA+ terminals with pleomorphic or flattened, small synaptic vesicles, probably belonging to axons of I-cells and/or thalamic reticular nucleus neurons. In addition to these classes of triads, it was observed that the retinal terminals established multiple synaptic contacts with both P-cell and I-cell dendrites, so that two types of arrangements with retinal input were recognized: the "simple" unit, frequent in parvocellular laminae, in which the retinal axon was accompanied by only I-2 presynaptic dendrites; and the "complex" unit, found mostly in magnocellular laminae, characterized by the presence of up to eight I-cell elements. In the latter units, "closely packed" classical triads, with the three synaptic junctions in close proximity, coexisted with triads "à distance" where the synapses were distributed relatively far from each other. The coupling of "closely-packed" and "à distance" triads by presynaptic dendrites resulted in the formation of multiple triadic arrangements. Aided by NIH Grants NS22953, NS11631 and EY01867.


Direction selectivity in visual cortex may be generated via bidirectional inputs which are in spatiotemporal quadrature (the inputs fire a quarter cycle apart in both space and time). Temporal quadrature can be obtained with conduction delays or slow synaptic phenomena, but such mechanisms may work only at high temporal frequencies. Another source of temporal quadrature at low frequencies might be found in the responses of the geniculate inputs to cortex.

We characterised the preferred response properties of cells in the cat LGN. X and Y cells can be divided into lagged and non-lagged varieties. At low temporal frequencies, lagged cell phase lags by about a quarter cycle relative to non-lagged cells in response to center and cemner stimuli. Response phase decreases approximately linearly with temporal frequency, more rapidly for lagged than for non-lagged cells. Because of this, at 4-5Hz the phase difference between lagged and non-lagged cells averaged a half cycle, so that temporal quadrature holds only at low frequencies.

We simulated the responses in each direction of a cell which simply sums inputs from pairs of geniculate cells. Using averaged data from lagged and non-lagged X cells, the simulation shows direction selectivity from 0.2Hz to 3Hz. At higher frequencies, the increased phase difference between the inputs (interval of direction preference). However, lagged cells respond poorly at higher frequencies, so that little direction selectivity is actually present. In contrast, pairs of actual cells can be chosen which give strong direction preference. This was paired with SI it altered the responses to SI. Results were paired for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI in the presence of remote or "disturbing" stimuli. These showed that the direction selectivity can be generated by appropriate combinations of lagged and non-lagged geniculate inputs. Supported by EY00304 and EY00459.

545.21 EFFECTS OF BINOCULAR STIMULATION ON SPATIAL AND CONTRAST SENSITIVITY OF CAT LGN NEURONS. L. Tong, W. Gudde, N. Tumaio, P.D. Spear, and S. Heidenreich. Dept. of Psychology and Center for Neuroscience, University of Wisconsin, Madison, WI 53706.

Previous studies indicate that most cat LGN neurons respond to stimulation of the nondominant eye and that the responses are spatial-frequency selective. In the present study, we examined the effects of nondominant-eye stimulation on spatial and contrast sensitivity through transcleral microelectrode recordings. Supported by EY0634 and EY00459.

545.20 DIVISION OF CAT RETINAL W-CELLS INTO TWO FUNCTIONAL CLASSES BASED ON QUANTITATIVE ANALYSES OF THEIR RESPONSE PROPERTIES. M.A. McCall, A.J. Weber and L.R. Stanford. Wisconsin Center and Department of Comparative Biomedical Sciences, University of Wisconsin, Madison, WI 53706.

Transcleral microelectrode recordings were used to record the responses of retinal ganglion cells (n = 214) to computer-generated stationary and sine wave stimuli. Multivariate statistical analyses were then used to determine the response characteristics that could most reliably separate these neurons into discrete subpopulations. Initially, 24 response variables were entered into the analysis in an attempt to define those characteristics that best separated ganglion cells into functional subgroups. Of the 24 variables, 7 response attributes were found to account for the majority of the separation among groups. These 7 variables were used in a final cluster analysis to determine the magnitude of the different clusters and the mean for each variable within a cluster. Our analysis showed that quantitative analyses of ganglion cell responses can effectively subdivide these neurons into a least 4 significantly different populations. The most powerful descriptors of the 4 functional groups were found to be axonal conduction properties and the overall level of responsiveness to visual stimuli. Two of these clusters that resulted from this analysis corresponded to the well defined retinal X- and Y-cell classes. The two remaining groups differed from X- and Y-cells primarily on their generally lower responsiveness to visual stimuli and were separable from one another on the basis of their axonal conduction properties, background discharge rates, and the duration of their response to stimuli of standing contrast. Although cells in both of these most probably belong to the heterogeneous "W-cell" classification, the members of the two groups are clearly separable on the basis of their functional characteristics.

We are currently investigating the possibility that additional response variables will further reduce the variability within the 4 clusters that we describe here and the possibility that additional quantitative data will allow us to unambiguously define further subclusters of retinal ganglion cells. Supported by NIH grant EY 04977.


Recently we have reported that the introduction of a second stimulus (S2) on the boundaries of the visual field has often altered the effects of a stationary receptive field (CRF) modifies the responses of a geniculocortical neuron (LGN) to a stationary test stimulus (S1), positioned in the RF. The current study concentrated on the characteristics of the responses to S1 in the presence of S2 and compared for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI it altered the responses to SI. Results were paired for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI it altered the responses to SI. Results were paired for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI it altered the responses to SI. Results were paired for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI it altered the responses to SI. Results were paired for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with SI it altered the responses to SI.
546.1

In retina melatonin regulates photoreceptors disc shedding and phagocytosis, melanosome aggregation in pigmented epithelium and dopaminergic release. The aim of this study was to localize binding sites in the rabbit retina using in vitro receptor binding auto-radiography. Binding sites were labeled by incubating 8 µM solutions of rabbit eye cups (fixed in 4% paraformaldehyde and frozen in cryoprotectant) with 2-[125I]-iodomelatonin (150 µM) in 150 µM Tris HCl buffer (pH 7.4) containing 0.1% BSA at 25 °C for 1 h. The highest density of specific binding defined with the melatonin receptor agonist 6-chloromelatonin (3 µM) was found (in order of magnitude) over the retinal pigmented epithelium (RPE) (52%), inner plexiform (62%) and inner nuclear layers (49%) and, over the outer and inner (45%) segments of photoreceptors. The melatonin receptor antagonist, luzindole (3 µM) defined the highest density of specific binding over the inner plexiform layer (68%) and the RPE (32%). Thus specific 2-[125I]-iodomelatonin binding sites were mainly associated with the synaptic portion of the inner retina, consistent with the role of melatonin to regulate dopaminergic amacrine cells in rabbit retina. Supported by grants EY-02294 to CB and MH-42992 to MLD.

546.2
REGULATION OF MELATONIN PRODUCTION IN HUMAN RETINOBLASTOMA CELLS. J.J. Janes, M.E. Pierce, D. Barker, and J.S. Takahashi. Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Retinas of several vertebrate species have been shown to produce melatonin in a circadian fashion. In lower vertebrates, retinal melatonin biosynthesis is regulated by cAMP through a mechanism that requires protein synthesis. We have recently shown that Y79 human retinoblastoma cells produce melatonin in static culture. Melatonin release is enhanced by light-stimulated cAMP that elevate cAMP. We now report that protein synthesis inhibitors block forskolin-stimulated melatonin release. Cycloheximide (10µM-40µM) reduced forskolin-stimulated melatonin production by 40-80%, respectively. Anisomycin (10µM-40µM) inhibited melatonin by 50-90%, respectively. To investigate whether melatonin production is a general property of human retinoblastoma tumors, we have begun to extend our studies to other retinoblastoma cell lines. We have found that static cultures of WERI-Rb1 retinoblastoma cells also produce melatonin, and that release can be enhanced by forskolin, 8-BrcAMP and the phosphodiesterase inhibitor, IBMX. Taken together, these results suggest that cAMP stimulation of melatonin production in retinoblastoma cells requires protein synthesis, as has been found for other vertebrate retinas. Melatonin production may be a common feature of human retinoblastoma cells.

(Supported by F31 EY04780, NS56392, and the Welch Foundation.)

546.3
MELATONIN SUPPRESSES THE LIGHT-EVOKED RELEASE OF ENDOGENOUS DOPAMINE FROM RETINAS OF FROGS (XENopus LAEVIS). J.B. Lombardini and S.M. Liebowitz. Texas Tech University Health Sciences Center, Lubbock, TX 79430 and College of Ophthalmology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390.

The putative neuromodulator melatonin is considered a signal for several dark-adapted retinal processes, including suppression of DA release. To examine the role of melatonin in regulation of DA neurons in frog retina, eye cups prepared in the last hour of the light phase from X. laevis maintained on a 12 hr light:dark cycle were dark-adapted for 90 min then incubated for 1 h in experimental conditions. Dark adapted DA extraction from incubation medium was analyzed by HPLC-EC and found (in order of magnitude) over the retinal pigmented epithelium (28%), inner nuclear (27%), outer nuclear (16%) and inner nuclear (14%) segments of photoreceptors. The melatonin receptor antagonist, luzindole (3 µM) defined the highest density of specific binding over the outer plexiform layer (68%) and the RPE (32%). Thus specific 2-[125I]-iodomelatonin binding sites were mainly associated with the synaptic portion of the inner retina, consistent with the role of melatonin to regulate dopaminergic amacrine cells in rabbit retina. Supported by grants EY-02294 to CB and MH-42992 to MLD.

546.4

Addition of melatonin (10^-12 - 10^-9 M) to the media superfusing intact rabbit retinas, suppressed the K^+-evoked (40 mM) release of endogenous dopamine (DA) in a dose dependent manner, the minimum effective concentration was 10^-11 M. At 10^-7 M melatonin inhibited this release maximally (by 58%, p<0.01). Melatonin failed to affect the spontaneous or K^+-evoked release of retinal acetylcholine (ACh). Retinal tyrosine hydroxylase (TH) activity was estimated by measuring 1-DOPA accumulation following pretreatment of rabbits with NSD-1015 (100 mg/kg). Melatonin blocked K^+-induced activation of retinal TH activity by 28-48%, at concentrations as low as 10^-11 M. DA release, measured simultaneously, was also inhibited (by 75% at 10^-9 M, p<0.05). Melatonin did not inhibit TH activity in reserpine-pretreated animals. To determine whether melatonin acts directly on DA-releasing cells, we prepared dispersed retinal cells by enzymatically disrupting their synaptic connections. DA release from dispersed retinal cells was stimulated by high K^+ (40 mM), and melatonin-enriched medium (100 mM) suppressed this K^+ stimulated release by 56% (p<0.01). These observations show that melatonin, at low concentrations, is an effective inhibitor of endogenous retinal DA synthesis and release, and that the hormone may have a direct effect on DA-releasing amacrine neurons.

(Supported by EY-02294 to CB and MH-42992, NSF PYI DCB-8451642 and Searle Scholars 85-H-107)

546.5
INHIBITORS OF CALCIUM ION UPTAKE IN THE RAT RETINA: QUANTITATIVE ANALYSES OF THE DOSE-EFFECT RELATIONSHIPS. J.B. Lombardini and S.M. Liebowitz. Texas Tech University Health Sciences Center, Lubbock, TX 79430 and College of Pharmacy, University of Texas, Austin, TX 78711.

Taurine is an amino acid that plays important roles in maintaining both the structural integrity and function of the retina. Thus, the effects of taurine, taurine analogues, and their combinations were studied in the ATP-dependent Ca^2+ ion uptake system in a rat retinal membrane preparation. (S)-2-Amino-4-methyl pentanonesulfonic acid (TAPS), a cyclic taurine analogue, was demonstrated to be noncompetitive (K_i = 0.05 M) with respect to taurine. 1,2,3,4-Tetrahydroxyquinoline-6-sulfonicacid (THQ), a less potent inhibitor of ATP-dependent Ca^2+ ion uptake than TAPS, was also shown to be noncompetitive with taurine (K_i = 23.8 M). When TAPS and THQ were tested in a fixed ratio mixture of 1:25 the inhibitory effects are synergistic as shown by the median-effect equation. (S)-Aminotetrathydrothiophene-1,1-dioxide (ATS) and (S)-piperidine-3-sulfonic acid (PSA) are an agonist and partial agonist that demonstrated stimulatory effects on ATP-dependent Ca^2+ ion uptake. ATS and taurine induced the same maximal rates of change on Ca^2+ ion uptake, however, PSA was less potent than taurine. The combination of taurine + ATS was additive while the combination of taurine + PSA was synergistic. (Supported by NIH grant EY04780 and the Welch Foundation.)

546.6
EFFECTS OF SULPHUR CONTAINING EXCITATORY AMINO ACIDS AND NAAG ON 3H-ACh RELEASE FROM THE RABBIT RETINA J.R Cunningham and M.J. Neal. Division of Pharmacology, St Thomas's Hospital, London, SE1 7EH, UK.

Homocysteic acid (HCA), cysteic acid (CA), homocysteinesulphuric acid (HCSA) cysteine-sulphonic acid (CSA) and N-acetylaspargly-glutamate (NAAG) all increased the resting release of ACh from the retina of rabbits anesthetised with urethane. This increase in resting release was associated with a reduction in the light-evoked release of ACh. Except for NAAG, the b-wave of the evg was not significantly reduced. All five compounds were antagonized by PDA indicating that they were acting on excitatory amino acid receptors. In the rabbit retina NMDA acts as an antagonist at the bipolar cell in the mullerian amacrine cell synapse. NMDA antagonised the effects of HCA and HCSA but not CA, CSA, NAAG, glutamate or aspartate. Since homocysteic acid blocks exogenously added "bipolar cell transmitter" the present results suggest that of the compounds tested only HCA and HCSA are likely to be the bipolar cell transmitters at cholinergic amacrine cells.

Monoclonal antibodies to pigmented epithelium (RPE) may play a role in receptor mediated phagocytosis of photoreceptor outer segments (ROS). We developed immunoperoxidase to the RPE to explore this role, and to determine if photoreceptors contain an inherited retinal degeneration. Possible differences between antigens from the apical surface of RPE of normal (LE) and dystrophic (RCS) rats were assessed by using the polyclonal antibodies. The monoclonal antibodies were raised against two different regions of the apical RPE with 800 and 200 KD bands (200 and 500) that were different between normal and RCS rats. No significant differences were detected between normal and dystrophic proteins with this antisera.


Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of serotonin, a neurotransmitter and precursor of melatonin. Our studies on tryptophan hydroxylation in the retina and pineal of chickens suggest that the enzyme is regulated by circadian oscillators. Accumulation of 5-hydroxytryptophan, the tryptophan hydroxylation reaction product, after inhibition of its metabolism by clomipramine, was measured in an in situ tryptophan hydroxylation assay. For animals maintained on a 12 h dark: 12 h light cycle, greatest activity was observed 4-5 h into the dark phase; minimum activity occurred 4-8 hours into light phase. A 3-5 fold light-dark difference in activity was observed in retina, 2 fold in pineal. On immunoelectroretinography, the enzyme activity precedes the beginning of dark phase. The D2 agonist quipirone inhibits isolation of rat retina and the growth of dopaminergic neurons using 5-flanking sequences of the human PNT gene. Retinal tumors were induced and tumor-derived cells have been propagated in tissue culture for 68 passages. The cells have a doubling time of ~24 hours and can be propagated in tissue culture for over 60 passages. ~ 95% of the cells are T antigen positive by immunofluorescence. Time-lapse video of extensive neuritic processes. The cells have a doubling time of ~24 hours and can be propagated in tissue culture for over 60 passages. ~ 95% of the cells are T antigen positive by immunofluorescence. Time-lapse video of extensive neuritic processes.

546.10 CHARACTERIZATION OF TAURINE RECEPTORS IN RETINAL PIGMENT EPITHELIUM CELLS IN CULTURE. A.M. Lopez-Colome, R. Saldana and G. Fragoso, Instituto de Fisiologia Celular, UNAM, Apdo. Postal 70-600, 04510 Mexico, D.F.

Taurine is also concentrated in the retina, particularly in the rod outer segments (ROS) from which it is released by illumination. A close interaction between RPE and the retina has been demonstrated in phenomena such as ROS shedding and in the ionic regulation of the subretinal space. Since the action of taurine on RPE is mediated by a receptor interaction, we explored the presence of specific taurine receptors in membranes from primary cultures of RPE. Cell membranes were obtained following the classical procedures, and H-taurine binding was measured. A saturable, high-affinity binding of taurine was detected, with Kd = 237 nM and Bmax = 1.8 pmol/mg protein. Binding showed to be Na independent, higher at 37°C compared to 4°C, and inhibited most potently by glycine, followed by strychnine, bicuculline, GABA and 8-alanine. Binding was higher (specific > 50%) in frozen than in freshly obtained membranes, and similar at days 16 and 25 in vitro. Since these binding sites do not seem to correspond to uptake sites, it could be suggested that taurine could act as a messenger from the retina to the RPE through an interaction with the receptors we have described.

546.11 AN IMMORTALIZED LINE OF RETINAL NEURONS DERIVED FROM 06492; Sch. of Vet.Med., Univ. of Penn., Philadelphia, PA 19104.

Monoclonal antibodies against retinal neurons were derived from a subline of SV40-T antigen positive cells isolated from a lineage of rat retinal neurons derived from the external limiting membrane of the retina (E.L. Miller, S.P. Schneider, L. Raff, pers. comm.). These cells have a morphology and metabolism to serotonin, was measured as an index of in situ tryptophan hydroxylase activity. For animals maintained on a 12 h dark: 12 h light cycle, greatest activity was observed 4-5 h into the dark phase; minimum activity occurred 4-8 hours into light phase. A 3-5 fold light-dark difference in activity was observed in retina, 2 fold in pineal. On immunoelectroretinography, the enzyme activity precedes the beginning of dark phase. The D2 agonist quipirone inhibits isolation of rat retina and the growth of dopaminergic neurons using 5-flanking sequences of the human PNT gene. Retinal tumors were induced and tumor-derived cells have been propagated in tissue culture for 68 passages. The cells have a doubling time of ~24 hours and can be propagated in tissue culture for over 60 passages. ~ 95% of the cells are T antigen positive by immunofluorescence. Time-lapse video of extensive neuritic processes. The cells have a doubling time of ~24 hours and can be propagated in tissue culture for over 60 passages. ~ 95% of the cells are T antigen positive by immunofluorescence. Time-lapse video of extensive neuritic processes.


Regulation of serotonin N-acetyltransferase (NAT), a key regulatory enzyme in the biosynthesis of serotonin, was measured in glia-free, low-density retinal cell cultures prepared from embryonic day 6 chicks. Photoreceptors represented approximately 70% of the cells in the cultures, the remaining cells were multipolar neurons and apparently undifferentiated round cells. NAT activity in these cells was markedly stimulated by agents that increase intracellular cyclic AMP, such as forskolin, isobutyrylmethylxanthine, and 8-B cyclic AMP. Stimulation was blocked by inhibitors of cyclic AMP and protein kinase C. Elevated K+ also increases NAT activity by a mechanism that involves Ca2+ influx through dihydroxyrocyclohexylamino reverse gated channels, as the K+-evoked increase is prevented by nifedipine, the Ca2+ channel blocker, and potentiated by BAY K 8644. Elevated K+ also increased intracellular cyclic AMP in the cells, suggesting a possible mechanism for the effect on NAT activity.

Dopamine inhibited the increases of NAT activity elicited by forskolin and by K+. The blocked of dopamine receptor antagonist sulpiride or by pretreatment with peruris toxin. Dopamine also decreases K+-evoked cyclic AMP levels. Thus, dopamine appears to regulate NAT activity in these cell cultures through a D1 dopamine receptor - G protein - adenylate cyclase complex.
546.13 TRANSFERRIN BINDING IN EMBRYONIC NEURAL RETINAL CULTURES. A.G. Hyndman, Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

Transferrin may be important in the regulation of neural differentiation. In order to increase our understanding of transferrin binding, high affinity (on average range) transferrin (tf) binding in purified neural cultures from both E8 and E11 chick neural retina was examined. Plates were in 30 min at 37°C and 2 hr at 37°C. The analysis of tf high affinity binding sites at 37°C demonstrated the following: 1) tf binding in E8 cultures was 2-fold greater than in E11 cultures. 2) Binding activity can be increased by stimulating neurons in the presence of tf. 3) Maintaining cultures in the presence of tf for 24 h resulted in increased appearance of immature sites which were less effective at tf binding. Cultures maintained in the presence of 0.2M free iron increased tf binding in E8 cultures by approximately 25% and by 20% in E11 cultures. A high affinity transferrin binding at 4°C gave similar results. NSF BNS-8810277.

546.14 MEMBRANE CURRENTS OF XENOPOD LAEVIS OOCYTES INJECTED WITH RNA FROM RETINAS OF CARP AND MOUSE. Lawrence H. Pinto, Neurobiology & Physiology Dept., Northwestern University, Evanston, IL 60208. Akimichi Kaneko, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444 Japan. Cathy Boyce and Deborah Farber, Jules Stein Institute, UCLA, Los Angeles, CA 90024-1727.

We measured voltage-clamp currents 3-7 days after injection of total RNA from retinas of adult carp (N=24 oocytes) or 9-11 day old C57BL/6J mice (N=10). Cortical injection of NMDA (100 M) after injecting carp RNA (0.7 m M [Ca2+]o depolarization (holding voltage, -90 mV) produced an outward current that was attenuated by 20mM TEA, 1 mM Ca2+ or 500 mM DIDS. Thus, I(V), I(Ca) and I(Cl) flowed in both oocytes. For oocytes injected with mouse RNA, depolarization (Vh=-90 mV) also produced a DIDS-sensitive outward current, but in 40 mM barium methanesulfonate the current became inward for depolarization to -10 to -20 mV. An inward tail current flowed after offset of the depolarizing pulse with 0.7 mM [Ca2+]o; this current was larger for oocytes injected with mouse RNA than for uninjected oocytes. The amplitude of the tail current increased by increasing [Ca2+]o, and was decreased by substitution of Ba2+ for Ca2+ or addition of 400 mU DIDS. No TTX-sensitive currents were observed. Thus, I(V), I(Ca) and I(Cl) of larger amplitude flowed in oocytes injected with mouse retinal RNA than in those injected with carp RNA.


Conventional quantitative labeling of muscarinic ACh receptors in visual cortex with tritiated QNB was compared in 7 species of mammals: 2 marsupials and 5 placentals, including 2 species of prosiman primates.

The results show that the density of m,7 receptors in striate cortex vary as much as 3-fold among species. However, more than 97% of this variation can be accounted for by 3 visual parameters: type of retina, vision-duration, and diurnal cycle. Furthermore, these same 3 parameters account for 95% of the variation in receptor density in layer 4 and in the infragranular layers and for 97% in the supragranular layers. However, when the density of receptors in the supragranular layer is normalized in each animal by using its ratio with perirhinal (PH) density in layer 4 (i.e., 52/49), the three vision parameters lose their explanatory power, now accounting for less than 20% of the variation. Instead, "degree of kinship to Anthropoids" or "phyletic grade" accounts best for the remaining variation with a single sharp increase in supragranular density occurring among the marsupials and placental's regardless of whether the placentals are Primates and regardless of the other variations in morphological form of their visual system. Supported by NIH-HSNSC #887426.


In the cerebral cortex of several species the lectin from Vicia villosa (VVA) labels the surface of a subpopulation of GABA cells. In monkey striate cortex, VVA labels about 30% of all GABA cells (Mulligan et al., 89, Vis. Neurosci., 2:65). Although the largest GABA cells in each layer label with VVA, it is unlikely that the labeled cells constitute a single cell type. In this study we further characterize the VVA-labeled cells by double-labeling experiments and by intracellular injection of VVA-labeled cells in fixed and in situ preparations.

Single section double-labeling experiments using different colored chromogens showed that virtually all VVA-labeled cells were immunonegative when reacted with antibodies to GABA. In the monkey striate cortex, VVA labels about 30% of all GABA cells (Mulligan et al., 89, Vis. Neurosci., 2:65). Although the largest GABA cells in each layer label with VVA, it is unlikely that the labeled cells constitute a single cell type. In this study we further characterize the VVA-labeled cells by double-labeling experiments and by intracellular injection of VVA-labeled cells in fixed and in situ preparations, these same 3 parameters account for 95% of the variation in receptor density in layer 4 and in the infragranular layers and for 97% in the supragranular layers. However, when the density of receptors in the supragranular layer is normalized in each animal by using its ratio with perirhinal (PH) density in layer 4 (i.e., 52/49), the three vision parameters lose their explanatory power, now accounting for less than 20% of the variation. Instead, "degree of kinship to Anthropoids" or "phyletic grade" accounts best for the remaining variation with a single sharp increase in supragranular density occurring among the marsupials and placental's regardless of whether the placentals are Primates and regardless of the other variations in morphological form of their visual system. Supported by NIH grants E10128 and EY04536.


The subcellular distribution of the receptor complex was determined using monoclonal antibodies to either the a- or the B-subunit and immunocytochemical techniques. Both subunits were distributed similarly. Intracellular immunoreactivity was associated with the endoplasmic ret., Golgi system and multivesicular bodies, suggesting the synthesis, glycosilation and degradation of the receptor. Extracellular immunoreactivity was on the membrane of neuronal dendrites and spinous processes. Immunoreactivity appears to be uniform at presumed GABAAergic synaptic junctions and at the non-junctional plasma membrane. Immunoreactivity was highest in layer 4. Differences in the density of receptors express the receptor complex to different classes of neurons. Some GABA-containing cells, especially the large multipolar cells with widely spreading varicose dendrites were recovered, from injections in layers 5 and 6. In layers 2 to 4 small multipolar cells with smooth radial dendrites were recovered, while injections in layers 2 and 3 often revealed medium-sized cells with prominently vertical dendrites. Thus, although the population of VVA-labeled cells appears neurochemically homogeneous, distinct morphological subtypes exist.

Supported by NIH grants EY01288 and EY04536.

547.4 DOWN-REGULATION OF GABA NEURONS IN AREA 17 OF MONOCUALLY DEPRIVED ADULT MONKEYS. S.H.C. Hendry, J.L. Parise, A.A. Robertson, and E.G. Jones. Dept. of Anatomy, Univ. of California, Irvine, CA 92717, Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203 and Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Within occular dominance columns driven by a deprived eye, the number of neurons immunoreactive for GABA is reduced by one-half (Hendry and Jones, 1988, Neurosci. 170). We used immunocytochemical and radioimmunoassay techniques to determine whether GABA receptors for GABA are down-regulated in deprived adult monkey visual cortex. The distribution of GABA receptors was examined in adult macaques (M. fascicularis; M. mulatta) that were normal, had one eye removed or had one eye injected with tetrodotoxin (TTX). The highest density of receptors was immunostained with a monoclonal antibody to the GABA, complex in normal animals was in layers 1VCB, IVCB and III. Layer 1VCB was uniformly stained in deprived monkeys. A similar pattern of staining was seen in layers 2 and 3. Differences in the density of neurons immunoreactive for GABA were reduced by 25-30% in layer 1VCB of the deprived-eye cortex affected.

Quantitative autoradiography showed the density of receptors was reduced by 25-30% in layer 1VCB of the deprived-eye cortex affected. These data demonstrate that parallel reductions in GABA and GABAA receptors occur in the deprived eye columns. Supporting by NEI grants EY07093 and EY04352.
547.5

THE CALCIUM-BINDING PROTEIN PARVALBUMIN IN THE STRIATE CORTEX OF MACAQUE MONKEYS AND HUMANS.


We analysed the occurrence of parvalbumin (PV) in the primary visual cortex (VI) in both primates to detect similarities and differences in the organisation of VI. In both primates the distribution of parvalbumin - immunoreactive (PV-ir) neurons and terminal fields matches that seen with cytochrome C-oxidase. However, the band-like PV-ir terminal fields of layer 4A are missing in human. Layer 1 is devoid of PV-ir cells. In monkeys and we found a lower amount of neurons in human layer 2. Morphologically PV-ir neurons resemble smooth dendritic multipolar- and stellate cells. In human VI we find smaller cells in layers 4B, 4Ca, 5 and 6. Immunoelectron-microscopy of monkey VI reveals many PV-ir synapses of the symmetrical, but also some of the asymmetrical type. The white matter contains PV-ir axons, located in efferent and afferent pathways. We assume that PV supports crucial functions of highly specialized neurons during visual perception.

547.7

EFFERENT PROJECTIONS OF LAYER 4 IN TREE SHREW STRIATE CORTEX: EVIDENCE FOR PARALLEL PATHWAYS TO THE SUPERFICIAL LAYERS.


Layer 4 in tree shrew striate cortex is divided into two tiers separated by a cell sparse cleft. The upper tier, 4A, receives projections from OFF-center LGN neurons; the lower tier, 4B, receives projections from ON-center LGN neurons. We studied the projections of 4A and 4B using HRP and FB injections into the LGN. Our results show that each tier consists of two parts that differ in their pattern of projection to the superficial layers. Injections in the upper part of 4A or lower 4B label axons and terminal arbors in layer 3C. In contrast, injections in lower 4A or upper 4B label bundles of axons that pass through 4A and 3C to terminate in layer 3B. These anterograde patterns are consistent with patterns of retrograde transport. Injections in layer 3B label cells immediately adjacent to the cleft, while injections limited to 3C label cell bodies in upper 4A and lower 4B.

We conclude that these two distinct and parallel pathways convey ON and OFF information from layer 4 to layers 3B and 3C. Since the central region of layer 4 receives input from the contralateral eye, while the flanking regions receive binocular input, layers 3B and 3C may differ in the way they process the inputs from the two eyes.

547.9

TRANSCALLOSAKY PROJECTING NEURONS IN CAT VISUAL CORTEX.

Alan Peters and Bertram Payne, Department of Anatomy, Boston University Medical School, Boston, MA 02118.

A large number of neurons that had been filled following injection of HRP into the contralateral marginal gyrus were examined by light microscopy. The purpose was to find antennal neurons that did not have the features of either pyramidal or spiny stellate cells, in order to inhibit neurons project axons across the corpus callosum. Of several hundred neurons exhibiting a Golgi-like filling, five neurons that appeared to lack the features of spiny neurons were selected for study. Subsequently, retrograde transport of HRP was used to study interconnections of these two visual areas. Efferents of Ec were mainly observed in the intratelecephalic connections between the visual areas in birds. Supported by NIH grants EY06681 and EY07840.

547.10

INTRATELECEPHALIC CONNECTIONS BETWEEN THE VISUAL AREAS IN BIRDS (Columba livia).

T. Shinjii, W. Woodson*, H. J. Karten and J.B. Schmidt*, Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92039.

The two major visual pathways to the telencephalon in the avian brain are the thalamofugal pathway (geniculo-striate pathway of mammals) and the tectofugal pathway (neco-thalamo-extratralaphathway of mammals). The visual wulst is the primary telencephalic target of the thalamofugal pathway, and contains four distinct laminae (HA, HAI, HIS and HD). The telencephalic recipient of the tectofugal pathway is a non-laminated aggregate, the core region of the ectostriatum (Ec). Horseradish peroxidase (HRP) and Phaseolus vulgaris Leucoagglutinin (PHA-L) were used to study interconnections of these two visual areas. Efferents of Ec were mainly observed in the surrounding peri-extratralaphathal belt (Ep) with few projections to the neostriatum. The Ep projects to the lateral neostriatum intermediate (NIL), confirming the report of Ritchie and Cohen (1977). When HRP was injected into the Ec, retrogradely labelled cells were found in the anterior lateral portion of the hyperstriatum ventrale (HV) and the PHA-L, it was found that the visual wulst was found to project heavily to the HA, and moderately to the lateral neostriatum frontal (NFL), Ep, and Hvl. Retinofugal connections support the existence of distinct streams within the visual pathways. Supported by ONR 800014-68-K-0504 and NINCDS PHS NS24460-03.
Morphological features of Area 17 efferents to extrastriate cortex in the tree shrew ( Tupale belangerii ).

M. A. Sesma and P. Mayer, School of Optometry, University of Missouri - St. Louis, 63121, U.S.A.

We have been examining features of visual cortical connections in the tree shrew using a variety of anatomical methods. In the tree shrew, Area 17 has major projections to two extrastriate cortical areas, area 18 and the temporal dorsal area (TD). These projections are multiple and periodic and may arise from different populations of neurons in the supragranular layers of area 17. To evaluate the morphology of individual efferent axons and the cells of origin in area 17 we used injections of the lectin, phaseolus vulgaris leucoagglutinin (PHA-L) or WGA-HRP.

Electrophoretic injections of PHA-L or pressure injections of WGA-HRP were made in area 17 of anesthetized tree shrews and post-injection survival ranged from 1 (WGA-HRP) to 10 days (PHA-L).

Reconstructed axons projecting to area 18 and TD had long, relatively straight trajectories with little or no branching until arborizing at discrete sites. Axonal arborizations in area 18 and TD were distributed over the middle layers with a horizontal extent of 350-400µm. Some axons had several arbors separated by gaps of 200µm-300µm but were restricted to a single area. In both areas the arborizations had en passage boutons and clusters of terminal boutons. These axons originate from small to medium size pyramidal cells in supragranular layers of area 17. Those cells projecting to area 18 lie throughout the supragranular layers while those projecting to area TD are restricted to layers II-III.

These studies indicate that neurons in area 17 possess axons that arborize within several distinct zones in a single extrastriate area. It is likely that area 17 conveys different information to area 18 and area TD.

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